DERMATOPATHOLOGICAL STUDY OF BOVINE CUTANEOUS PAPILLOMATOSIS (WART) AND ITS MANAGEMENTAL APPROACHES

A THESIS

BY

RATAN KUMAR GHOSH

SEMESTER: MARCH, 2011 - AUGUST, 2011
REGISTRATION NO: 1005026
SESSION: 2010-2011

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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FEBRUARY, 2012

DEDICATED

TO
MY

BELOVED PARENTS

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The author

February, 2012

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ABBREVIATIONS AND SYMBOLS USED IN THIS TEXT

%	: Percentage
° C	: Degree centrigrade
g	: Gram
ml	: Milliliter
hr	: Hour
hrs	: Hours
H & E	: Haematoxylin and eosin
nm	: Nanometer
No.	: Number
&	: and
VTH	: Veterinary Teaching Hospital
HSTU	: Hajee Mohammad Danesh Science and Technology University
VTH-HSTU	: Veterinary Teaching Hospital of Hajee Mohammad Danesh Science and Technology University
VAS	: Veterinary and Animal Science
/	: or
et al	: and his associates
A.D	: (*anno domini*) in the year of the lord
ULO	: Upazila Livestock Officer
DNA	: Deoxyribonucleic acid
dsDNA	: double stranded Deoxyribonucleic acid
PV	: Papillomavirus
BPV	Bovine papillomavirus
LCR	: Long control region
ORFs	: Open reading frames
RPA	: Replication protein A
PCR	: Polymerase chain reaction
pRb	: Retinoblastoma protein
p53	: Protein 53 or tumor protein 53
PML	: Promyelocytic leukemia protein
E_1	: Early region (non structural protein)
L_1	: Late region (structural protein)

ABSTRACT

Dermatopathological study of bovine cutaneous papillomatosis (wart) and its multidimensional therapeutic approaches was carried out at different regions of Dinajpur district. The course of the study was one year from March, 2011 to February, 2012 and the laboratory examination was done at Pathological Laboratory, Department of Pathology and Parasitology, HSTU. The animals submitted to the VTH-HSTU for diagnosis and treatment and treated during physical visit were considered as experimental animals. The total clinical cases were 886, among which only 12 cases were typically affected with papillomatosis. The clinical signs, gross morbid lesions, topographic position of lesions and the general health status of suspected animals were recorded. The skins of 3 typically papillomatosis affected animals were collected surgically with required precautions and normal skin from slaughter house were also collected, preserved, processed for histopathological study. Affected animals were divided into 4 groups (Group A, B, C, D) received autogenous vaccine, autohemotherapy, both autogenous vaccination and autohemotherapy and surgical excision, respectively. The animals were clinically characterized as poor health status, reduced productivity, grossly as pedunculated cauliflower like, sessile, pea or bean shaped and flat type of outgrowth over skin and histopathologically characterized by hyperkeratosis, acanthosis, hypergranulosis, downgrowth rete ridges and neoplastic cells islands. Among the four therapeutic groups, Group D shows better therapeutic response although having some limitations.

CHAPTER I

INTRODUCTION

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INTRODUCTION

Papillomatosis is a chronic proliferative disease, caused by a DNA viruses belonging to the family *Papovaviridae* (Fonner *et al.,* 1974). Bovine papillomatosis is a common viral disease of the skin, mostly of young cattle, manifested as benign tumours or warts, caused by bovine papillomavirus (BPV) that has six serotypes hitherto described (Olson, 1990). Papillomaviruses are small (55 nm in diameter) non enveloped, icosahedral viruses, containing a double stranded, circular DNA genome about 8000 base pairs long. Bovine papillomatosis (warts) is a disease caused by host, site and lesion specific papillomaviruses (Nasir and Campo, 2008). They are classified as mucosotropic or cutaneotropic tropism (Souto *et al.*, 2005).

Papillomaviruses induce the development of localized proliferative lesions of the skin and mucous in a wide range of hosts (Le Net *et al.*, 1997). They are found throughout higher vertebrates, mostly mammals and birds, causing cutaneous and mucosal tumours (William, 2009). Papillomavirus infection has also been detected in genital and cutaneous lesions of several species of marine mammals (Van Bressem *et al.*, 1996; Kennedy-Stoskopf, 2001; Bossart *et al.*, 2002) and the healthy skin of humans and animal species can harbor sub-clinical PV infections in the absence of overt lesions (Antonsson *et al.*, 2000; Antonsson and Hansson, 2002).

Their oncogenic potential is related to the viral proteins E6 and E7, which are capable of interacting with proteins that regulate the cellular cycle and act as tumor suppressors. This interaction induces an uncontrollable regulation of the cellular cycle, causing the neoplasics formation (Souto *et al.*, 2005). The initial infection by PV occurs in the basal layers. These basal cells differ and move forward in the direction of the epithelial surface layers. The production of PV is restricted to the suprabasal cells, where the daughter cells in the basal layer are not broken by the production of new infectious viral particles and continue proliferating as reservoir of viral DNA for future cellular divisions (Souto *et al.*, 2005).

These viruses have been found to have substantial clinical importance (Syrjanen *et al.*, 1987; Zur Hausen, 1991). The spread of the disease is usually via direct contact, contaminated food and

equipment, castration and injections. Inheritance, nutritional and hormonal disorders, sunlight and suppressed immune system may play important roles in pathogenesis of disease (Theilen *et al.*, 1985; Campo *et al.*, 1994; Dinc, 1995; Nicholls *et al.*, 2000; Otter and Leonard, 2003). It gains its economic importance through interfering with animal sales and shows, as extensive bovine papillomatosis causes the animal to lose his condition especially when the lesions get infected secondarily with bacteria. Teat warts are also interfering with milking process (Radostitis *et al.*, 2007). These warts may regress spontaneously or occasionally persist, and, in the presence of additional critical genetic or environmental factors, can progress to cancer (Campo, 1987). It is thought to be a multistep affair (Koller and Olson, 1972; Lancaster and Olson, 1982). Papilloma virus infection developed as a result of the virus exposure to single or multiple lesions of the epithelium of the skin. The transformation and multiplication of papilloma virus infected basal cells, lead to wart formation, the most warts are benign and do not proliferate indefinitely causing cancer (Shah and Howley, 1996). The immune-suppressive factors play a role in progression of bovine papillomatosis (Radostitis *et al.*, 2007), including internal and external parasites. Warts are solid outgrowth of epidermis and it may be single or multiple and may be sessile or pedunculated have been reported by different authors (Radostits *et al.*, 1944). Histopathologically, the bovine cutaneous papillomatosis is described as viral cytopathic effect known as Koiliocytosis, considered being the "larger criterion" in the *papillomavirus* infection (Xavier *et al.*, 2005).

Although cutaneous papillomatosis do not cause death of the animal but chronic illness with generalized warts lead to heavy economic loss due to unthriftiness, damage of the skin, reduced market value of affected animals, and poor working quality of draught animals. In addition, sometimes rubbing, bleeding myiasis and sepsis are reported to be associated with this disease (Soni and Parekh, 1977, Prasad *et al.*, 1988).

Different methods have been used to treat bovine papillomas. Formalinized inactivated vaccine of bovine warts proved to be effective treatment and good prophylaxis against bovine papillomatosis (Barthold *et al.*,1976; Hunt, 1984; Lesnik *et al.*,1999; Suveges and Schmidt, 2003). Intra-lesional immunotherapy by *Corynebacterium parvum* has also been reported (Hall *et al.*, 1994). Surgical excision, anthiomaline injection and autogenous vaccine have been reported by many workers as the effective therapeutic measures against bovine cutaneous papillomatosis

in Bangladesh (Mia and Haque, 1967, Ahmed *et al.,* 1978). Local application of a paste containing arsenic trioxide 12%, arsenic trisulphide 6%, Zinc chloride 3% and benzocaine 0.7% (Lindley, 1974) have been tried against bovine cutaneous papillomatosis elsewhere with variable results. From the above facts it is quite evident that the disease under consideration is of much economic importance which needs further detailed study. Therefore, considering the facts and findings as stated above, the present study was undertaken with following objectives:

i) To study the clinical as well as gross morbid features of warts and their topographic distribution on animal body.
ii) To study the histopathological features containing typical lesions of papillomatosis affected skins.
iii) To observe the therapeutic responses following multidimensional therapeutic approaches.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1. OETIOLOGY

In cattle BPVs (Bovine papillomaviruses) induce benign tumours of cutaneous or mucosal epithelia, called papillomas or warts (Nasir and Campo, 2008).

2.2. TAXONOMIC POSITION OF THE OETIOLOGIC AGENT

Group: Group 1 (dsDNA)

Order: Unranked

Family: Papillomaviridae

Genus: *Deltapapillomavirus, Epsilonpapillomavirus, Xipapillomavirus* (De Villiers *et al.*, 2004)

The papillomaviridae family comprises 16 virus genera classified based on genomic DNA homology, especially of the late L1 structural protein gene (Alfieri *et al.*, 2008). Suspensions of bovine cutaneous papilloma contain an agent capable of producing more than 1 type of neoplastic reaction in a variety of tissues of several different species of animals. This agent has been identified as the bovine cutaneous papilloma virus (BPV) (Smithies and Olson, 1961; Brobst and Hinsman, 1966; Fujimoto and Olson, 1966).

Six types of BPV have been characterised, BPV-1 to BPV-6 (Pfister *et al.*, 1979; Campo *et al.*, 1980, 1981; Campo and Coggins, 1982; Chen *et al.*, 1982; Jarrett *et al.*, 1984; Olson, 1990; Alfieri *et al.*, 2008), which are divided into three broad subgroups. Some author says while hundreds of papillomavirus (PV) types have been described in humans, only ten bovine papillomavirus (BPV) types (BPV-1 to -10) are recognized (De Villiers *et al.*, 2004; Ogawa *et al.*, 2007; Tomita *et al.*, 2007; Hatama *et al.*, 2008).

Table 1: Distribution of lesion by type of virus (Hunt, 1984; Claus *et al.*, 2007; Alfieri *et al.*, 2008; Nasir *et al.*, 2008;)

Genus	Virus strain	Usual site
Deltapapillomavirus or fibropapillomaviruses	BPV-1	Infects paragenital areas, including penis, teats and udders (Ogawa *et al.*, 2004; Claus *et al.*, 2007; Alfieri *et al.*, 2008; Nasir *et al.*, 2008).
	BPV-2	Infects skin, alimentary canal and urinary bladder. (Claus *et al.*, 2007; Alfieri *et al.*, 2008; Nasir *et al.*, 2008).
Xipapillomavirus or epitheliotropic BPV	BPV-3	Infects skin. (Claus *et al.*, 2007; Alfieri *et al.*, 2008; Nasir *et al.*, 2008).
	BPV-4	Infects the upper alimentary tract. (Campo *et al.*, 1994; Borzacchiello *et al.*, 2003; Claus *et al.*, 2007; Alfieri *et al.*, 2008; Nasir *et al.*, 2008).
	BPV-6	Infects teat and udder. (Ogawa *et al.*, 2004; Claus *et al.*, 2007; Alfieri *et al.*, 2008; Nasir *et al.*, 2008).
Epsilonpapillomavirus	BPV-5	Infects teat and udder. (Claus *et al.*, 2007; Alfieri *et al.*, 2008; Nasir *et al.*, 2008).

In addition, the most recently characterized BPV types were placed in *Epsilonpapillomavirus* (BPV-8) and *Xipapillomavirus* (BPV-9 and -10), with the exception of BPV-7, which belongs to an undesignated PV genus (Ogawa *et al.*, 2007; Tomita *et al.*, 2007; Hatama *et al.*, 2008).

2.3. PROPERTIES OF VIRUS

Like other papillomaviruses, BPVs are small non-enveloped viruses with an icosahedral capsid around 50–60 nm in diameter (Shah and Howley, 1996; Campo, 2006). The capsid is formed of the L1 and L2 structural proteins (Shah and Howley, 1996; Modis *et al.*, 2002). The genetic organisation of those BPVs which have been sequenced is broadly similar to other papillomaviruses. The open reading frames (ORFs) are all located on one strand, and are divided into early and late regions. The early region encodes nonstructural proteins E1 to E7. There are three viral oncoproteins, E5, E6 and E7; BPVs of the *Xipapillomavirus* group lack E6. The late region encodes structural proteins L1 and L2. There is also a non-coding long control region (LCR) (Shah and Howley, 1996).

The papillomavirus contain double-stranded cyclic DNA with a mass of about 5 x 10^6 daltons, which is sufficient to code for about 300000 daltons of protein (Lancaster and Olson, 1981). Up to 10 polypeptides can be identified from papillomaviruses. The major polypeptides range in size from 50000 to 63000 daltons (Spira *et al.*, 1974; Lancaster and Olson, 1978).

Immunological cross-reactivity does not occur between papillomaviruses of different animal species, although these viruses apparently do share a common internal antigen (Jenson *et al.*, 1980). Several of them hemagglutinate erythrocyte by reacting with neuraminidase-sensitive receptors.

Papillomaviruses do not reproduce readily in cell cultures (Lancaster and Olson, 1981; Taichman *et al.*, 1984); consequently, details of their replication are sketchy. Papillomaviruses have a predilection for epithelial cells and usually infection is not only species-specific but also restricted to certain defined epithelial tissues (Smith and Campo, 1985).

The virus is resistant to chloroform and remains viable for 180 days at -70° C. and 90 days at 4° C. (Merchant & Packer, 1967).

2.4. PAPILLOMAVIRUS REPLICATION CYCLE

Key life cycle events seem to be similarly regulated in both human and non-human PVs (Peh *et al.*, 2002). The PV life cycle is strictly dependent on differentiation of the epithelial tissue (Barksdale and Baker, 1993) and PV replication can be divided into three stages (McBride *et al.*, 2000).

First, the PV virion must bind to a basal keratinocyte, although studies have shown that the PV virions can bind a wide variety of cell types (Muller *et al.*, 1995). During this stage, the viral genome is maintained as an episome within the nucleus (McBride *et al.*, 2000). The viral genome is then amplified and the viral copy number is increased up to 1,000 per haploid cell genome (Lepik *et al.*, 1998). As the basal cells differentiate, the viral DNA is maintained as a stable plasmid (Howley and Lowy, 2001).

During this second, maintenance stage, the viral genome replicates in synchrony with the host cell chromosome (Gilbert and Cohen, 1987). The PVs rely on cellular replication factors and enzymes (Muller *et al.*, 1994), such as replication protein A (RPA) (Mannik *et al.*, 2002), in order to replicate their genomes from a single origin of replication (Melendy *et al.*, 1995). The earliest PV DNA synthesis is within the fragment containing the PV replication origin and synthesis proceeds in both directions from the replication origin (Melendy *et al.*, 1995).

The third replication stage takes place in the terminally differentiated epithelial cells of the papilloma (Howley and Lowy, 2001). In this next layer of stratified epithelium, the stratum granulosum, late viral gene expression, synthesis of capsid proteins, vegetative viral DNA synthesis, and assembly of virions occur (McBride *et al.*, 2000). The PV DNA is thought to remain in the basal epithelial cells and to be reactivated when levels of immune system monitoring decline (Doorbar, 2005).

2.5. HISTORY AND DISTRIBUTION

Warts in animals have been recognized for centuries. Equine papillomas were described as early as in the 9th century A. D. and the first experimental transmission of animal papillomas occurred in 1898 (Lancaster and Olson, 1982). Warts in wild cottontail rabbits were the first animal papillomas thoroughly examined for properties of transmissibility, etiology, and histology. The activities and characteristics of the papilloma-producing agent in cottontail rabbits classified it as a virus (Shope, 1933). Additional non-human PVs initially characterized include: BPV (Lancaster and Olson, 1978), equine PV (Fulton *et al.*, 1970), canine oral PV (Chambers and Evans, 1959), deer fibromavirus (Shope *et al.*, 1958), and chaffinch PV (Lina *et al.*, 1973). Presently, 22 animal PVs have been fully characterized and classified into genera and species based on the L1 ORF sequences (de Villiers *et al.*, 2004). As many as 53 putative new animal PV types have been identified by polymerase chain reaction (PCR) in 7 animal species, including chimpanzees, gorillas, spider monkeys, long-tailed macaques, domestic cattle, aurochs and European elk (Antonsson and Hansson, 2002).

Cattle warts apparently occur in all countries. Royere, in 1902, was able to transmit experimentally the warts of cattle, horses and dogs. Magalhaes, in 1920, was able to transmit cattle warts by using Berkefeld filtrates of wart material (Merchant and Packer, 1967). By using specific primers, BPV type 1 was described in skin warts, peripheral blood and plasma from cattle with cutaneous papillomatosis, while BPV type 2 was detected in whole blood and urinary bladder tumors from cattle with chronic enzootic haematuria and cutaneous papillomatosis (Santos *et al.*, 1998; Freitas *et al.*, 2003; Wosiacki *et al.*, 2005, 2006). Recent studies employing PCR with generic primers in combination with cloning and sequencing, have described 15 putative new BPV types (Forslund *et al.*, 1999; Antonsson and Hansson, 2002; Ogawa *et al.*, 2004). After characterization of their complete genome sequences, four of these Japanese isolates were recently recognized as new viral types (BPV-7, -8, -9, and -10) (Ogawa *et al.*, 2007; Tomita *et al.*, 2007; Hatama *et al.*, 2008). In addition, four putative new BPV types have been identified in cutaneous lesions from cattle herds in Parana state, Brazil (Claus *et al.*, 2008). BPV-8 was first detected from papillomas as well as healthy teat skin from cattle in Japan (Claus *et al.*, 2008). In addition, a variant of BPV-8 was detected in papillomatous lesions of a European bison from

Slovakia, demonstrating that this new BPV type was present, simultaneously, in Asia and Europe (Literák *et al.*, 2006; Tomita *et al.*, 2007).

2.6. HOST SPECIFICITY

Papillomaviruses are the cause of cutaneous warts in cattle and horses. These viruses have considerable host specificity. In cattle, warts can occur on almost any part of the body. These warts are often morphologically specific, caused by distinct papillomaviruses, so that immunity to one of them does not necessarily confer immunity to others (Berrier, 2002). The papillomaviruses, which are widely distributed in nature, typically induce benign skin tumours (warts) in their natural hosts (Orth *et al.*, 1977). Some bovine papilloma viruses (BPV) are exceptional in that they induce fibropapillomas in their natural host (cows); these BPV can also induce cellular transformation in tissue culture (Black *et al.*, 1963; Boiron *et al.*, 1964; Thomas *et al.*, 1964) and fibroblastic tumours in heterologous animals, including hamsters (Friedman *et al.*, 1963), mice (Boiron *et al.*, 1964) and horses (Olson *et al.*, 1969; Lancaster *et al.*, 1977).

Bovine papovaviruses (BPV-1 and BPV-2) can cause various mesenchymal neoplasms when injected into hamsters and calves (Cheville, 1966; Gordon and Olson, 1968; Olson *et al.*, 1969; Robl and Olson, 1968; Stannard and Pulley, 1978) and can cause fibroblastic skin tumours in horses but are not pathogenic for goats and sheep (Gibbs, 1975; Abu-Samra, 1982). Ovine papovavirus pathogenic for hamsters but not for cattle and goats (Gibbs, 1975).

2.7. AGE SUSCEPTIBILITY

The disease affects cattle of all ages but mostly young animals (Olson, 1993; Smith, 1996; Imren and Sahal, 1997; Nicholls and Stanley, 2000). The occurance of cutaneous papillomatosis (warts) in cattle is mostly under 2 years of age (Creech, 1929; Liess, 1934; Jarmai, 1937; Bagdonas and Olson, 1954; Champawat *et al.*, 1986). Older cattle and horses appeared to be somewhat resistant to the infection, since experimental transmission or exposure to older animals failed to produce the disease (Jarmai, 1937; Boley, 1940).

2.8. SOURCE OF INFECTION

Cattle are the main source and natural reservoir of infection by the virus; but, halters, ropes and instruments can serve as a potential source of infection (Olson *et al.*, 1993; Araibi *et al.*, 2004; Lindsey *et al.*, 2009).

2.9. MODE OF TRANSMISSION

The method of spread is by direct contact with infected animals, infection gaining entry through skin abrasions (Berrier, 2002). The viruses have the ability to penetrate cells by different mechanisms leading to chromosomal instability and some of them contribute to the development of cancer cells (Duelli and Lazebnik, 2007). Direct contact is the main mode of transmission although indirect factors such as contaminated food and equipment, castration, injections and the use of immune-suppressants may also be implicated (Campo *et al.,* 1994; Dinç, 1995; Nicholls and Stanley, 2000; Otter and Leonard, 2003). The wart lesion appeared to be transmitted from one animal to another and also hands of the worker of the farm (Frenz, 1941). Another rather common bovine papilloma, seen on the end of penis of bulls and in the vagina of cows, may be spread during breeding (McEntee, 1950).

2.10. IMMUNITY

Spontaneous remission of papillomas is reportedly uncommon, and regression of papillomas may take almost a year (Olson *et al.*, 1992; Campo *et al.*, 1994; Smith, 1996). After several months, the lesions of bovine papillomatosis usually regress spontaneously, presumably from a gradual development of an effective immune response. Once cattle eliminate these skin warts they appear to be resistant to infection. However, the incidence of cutaneous warts on the teats in older cattle appears to increase with age (Blood *et al.,* 1983; Meischke, 1979). Although benign papillomas typically undergo spontaneous regression, some do not, and some progress to become malignant epithelial tumours (Campo *et al.,* 1994; Nicholls and Stanley, 2000). The regression of lesions is probably affected by cellular rather than humoral immunity (Nicholls and Stanley, 2000).

2.11. ZOONOTIC IMPORTANCE

Early literature suggested possible transmission of bovine warts to humans, but recent investigations indicate that human and bovine viruses do not cross-infect (Lancaster and Olson, 1981).

2.12. FACTORS AFFECTING FOR THE DEVELOPMENT OF LESION

The immune suppression enhance papilloma virus infection (Lesnik *et al.*, 1999; Brady *et al.*, 1999; Koski and Scott, 2003). The immune-suppressive factors play a role in progression of bovine papillomatosis are internal and external parasitism (Radostits *et al.*, 2007). It is believed that ticks have two inducing roles for bovine papillomatosis viz-

a) They pierce the skin and create entry points where viruses enter the cutaneous tissue, infect basal keratinocytes and replicate their genomic materials in the differentiating spinous and granular layers of the epidermis, and cause development of excessively grown warts (Radostits *et al.*, 2007).

b) The tick sucks a large volume of host blood where it inserts its hypostome into the skin and secretes cement from the salivary glands to secure the hypostome in place. They damage the skin barrier while feeding on host blood; secrete saliva that prevent the clotting of blood and has also immune suppressive effects (Salib and Farghali, 2008).

Deficiencies of iron, molybdenum, copper and zinc have been associated with higher worm burdens consequently affected immune response (Koski and Scott, 2003). Papillomavirus infection in cattle could be connected with serious disorders of the metabolism (mainly mineral, energetic and nitrous) probably caused by damage of the liver and kidney with mutagenic, carcinogenic and immunosuppressive cadmium, arsenic and lead, observed in the serum of tested animals (Lesnik *et al.*, 1999).

Exposition of cattle in bracken fern (*Pteridium aquilinum*) ingestion can be considered a cancer agent which interferes in the early or late levels carcinogenesis process acting like a cofactor in papillomavirus infection. Bracken fern contains immunosuppressant and mutagen substances that probably induce chromosomal abnormalities (Leal *et al.*, 2003).

2.13. PATHOGENESIS

2.13.1. Infectious entry

Papillomaviruses gain access to keratinocyte stem cells through small wounds, known as microtraumas, in the skin or mucosal surface. Interactions between L1 and sulfated sugars on the cell surface promote initial attachment of the virus (Joyce *et al.*, 1999; Giroglou *et al.*, 2001). The virus is then able to get inside from the cell surface via interaction with a specific receptor, likely via the alpha-6 beta-4 integrin (Evander *et al.*, 1997; McMillan *et al.*, 1999), and transported to membrane-enclosed vesicles called endosomes (Selinka *et al.*, 2002; Day *et al.*, 2003). The capsid protein L2 disrupts the membrane of the endosome, allowing the viral genome to escape and traffic, along with L2, to the cell nucleus (Kamper *et al.*, 2006; Day *et al.*, 2004).

2.13.2. Viral persistence

After successful infection of a keratinocyte, the virus expresses E1 and E2 proteins, which are for replicating and maintaining the viral DNA as a circular episome. The viral oncogenes E6 and E7 promote cell growth by inactivating the tumor suppressor proteins p53 and pRb. Keratinocyte stem cells in the epithelial basement layer can maintain papillomavirus genomes for decades (Doorbar, 2005).

2.13.3. Production of progeny virus

The expression of the viral late genes, L1 and L2, is exclusively restricted to differentiating keratinocytes in the outermost layers of the skin or mucosal surface. The increased expression of L1 and L2 is typically correlated with a dramatic increase in the number of copies of the viral genome. Since the outer layers of stratified squamous epithelia are subject to relatively limited

surveillance by cells of the immune system, it is thought that this restriction of viral late gene expression represents a form of immune evasion.

New infectious progeny viruses are assembled in the cell nucleus. Papillomaviruses have evolved a mechanism for releasing virions into the environment. Other kinds of non-enveloped animal viruses utilize an active lytic process to kill the host cell, allowing release of progeny virus particles. Often this lytic process is associated with inflammation, which might trigger immune attack against the virus. Papillomaviruses exploit desquamation as a stealthy, non-inflammatory release mechanism. The L1 and L2 proteins are assembled late and spontaneously form icosahedral capsids. Following virion assembly, mature viruses are released from the uppermost layers of the epithelium (Hummel *et al.*, 1992).

2.13.4. Production of lesions

Warts will appear 1 to 6 months after inoculation with the virus (Morter and Horstman, 2007). Lesion in the natural cases are categorized into three phases; growth, development and regression. Main lesions of the growing phase are marked hyperplasia of the basal cells and mild to moderate acanthosis, hyperkeratosis with a few intranuclear inclusion bodies which are positive with antibovine papillomavirus serum (Hamada *et al.*, 1990).

In the developing phase, there is prominent acanthosis with cellular swelling and fusion occurs (Eisa *et al.*, 2000). Many intranuclear inclusion bodies are also present in swollen or degenerative prickle cells and granular cells.

In the regression phase, epidermal layers are almost normal with only slight hyperplastic changes (Hamada *et al.*, 1990; Hamada *et al.*, 1992). It has long been assumed that papilloma regression is mediated by immunological mechanisms which are probably cellular in nature (Hall *et al.*, 1994).

2.14. CLINICAL SIGN

Infection by bovine papillomavirus (BPV) causes cutaneous papillomatosis and benign proliferative lesions that can result in severe injuries and losses in animal production (Jelinek *et al.*, 2005).

In cattle, warts on the teats, penis or interdigital skin or in the alimentary tract may produce clinical signs of pain or occlusion (Rebhun, 1980). Papilloma or warts are occasionally seen in cattle and buffalo. The warts do not disturb the general body function, but sometimes associated with health hazard due to rubbing, bleeding, sepsis and myiasis (Soni *et al.*, 1977). The more usual sites of the lesions occur in the neck, shoulder, costal, dewlap, forelegs and face region (Bagdonas and Olson, 1953; Das, 1982). The general health condition of cattle is being deteriorated (Rahman *et al.,* 1969). Ante-mortem defects of the disease not only affect the quality of leathers but also affect the health of the live animals (Dey and Nooruddin, 1992). Large warts are subjected to trauma leading to haemorrhage and secondary infection resulting in a necrotic dermatitis (Huck, 1965). Teat warts are interfering with milking process (Campo, 2006; Radostits *et al.*, 2007). Fibropapillomas can be troublesome when present in the genital area, causing pain and sometimes loss of reproductive functions as well as interfering with calving (Merck, 1986; Campo, 2006). In chronic infections some animals may lose condition, be stunted and very rarely death may occur. Chronically immunosuppressed animals may develop extensive papillomatosis in the upper gastrointestinal tract, which can cause difficulties with eating and breathing (Campo, 2006). Some of the lesions regress spontaneously but others prove refractory to treatment (Beutner and Ferenczy, 1997). If warts occasionally persist and in the presence of additional critical genetic or environmental factors, can progress to cancer (Campo, 1987).

2.15. GROSS LESION

Papillomatous lesions varying degrees in sizes and shapes, disseminated on the ears, head, neck, shoulders, abdomen, udder and perigenitaly (Turk *et al.*, 2005).

The four most common types of warts are squat (sessile), pedunculated (stalked), flat and tags. They appear as raised hairless lesions (varying in size from a pea to a tennis ball).

Warts caused by the *Xipapillomavirus* group have a cauliflower-like appearance and can attain the size of a fist; most common on the head, neck and shoulders, they may also occur in other locations. Cutaneous fibropapillomas caused by *Deltapapillomavirus* group have a nodular appearance (Merck, 1986).

Tumour tissue composed of hyperplastic epidermis supported by thin, inconspicuous dermal stalks (Turk *et al.*, 2005).

Ocular papillomas occur in range cattle and may be precursors of squamous cell carcinoma (Ford, 1982; Hunt, 1984). Lesions may be long and frond like or round and broad-based and tend to be attached to the eyelid or corneoscleral junction (Scott, 1988). BPV has not been recovered from these lesions, but virions resembling papovavirus have been detected by electron microscopy (Ford, 1982; Hunt, 1984).

2.16. MICROSCOPIC LESION

There is marked parakeratotic hyperkeratosis with long, thick hair-like cornified surface projections and papillate epidermal hyperplasia with patchy areas of erosion, ulceration and neutrophilic infiltration occur (Salib and Farghali, 2008). The underlying dermal papillae have a moderate infiltration of neutrophils, eosinophils and fewer lymphocytes (Edward, 1994).

Histopathologically there is fibropapillomatosis with acanthosis, hyperkeratosis and down-growth of rete ridges found. The virus appears to infect the basal cells of the epithelium, causing hyperplasia with hydropic ballooning of their cytoplasms, large eosinophilic keratohyaline granules and vesicular nuclei. Some cells degenerated, while others stimulated to excessive growth and formation of warts (Turk *et al.*, 2005). The stratum spinosum, in particular is markedly hyperplastic with most cells containing multiple, variable sized and shaped (Bloch *et al.*, 1994).

The koilocytosis was described initially as cells with nuclei picnotics, moderately irregular, outlined by extensive clear halos with superior volume than the cytoplasm (Silveira *et al.*, 2005). The disceratosis occurs together with the koilocytosis and it consists densely of the premature keratinization in cytoplasm form densely eosinophilic, with opaque nucleus, hyperchromatic and irregular (Silveira *et al.*, 2005). Other authors affirm that the koilocytosis constitutes a sign of pathognomonic infection by *papillomavirus* (Xavier *et al.*, 2005).

Electron microscopic images of the wart suspensions indicated a small non enveloped virus with an approximate diameter of 60 nm, composed of capsomeres arranged in icosahedral symmetry. The virus description matched that of BPV as previously reported (Shah and Howley, 1996).

2.17. DIAGNOSIS

The diagnosis of bovine papillomatosis is usually made from the presenting clinical signs because the structure of the papillomas or fibropapillomas on the skin or mucosal membranes is easily observed and identified (Hunt, 1984).

The diagnosis can be confirmed by biopsy and histopathological examination of tissues (Marins and Travassos, 2011).

The viral type involved can be determined by serologic assays for specific antibody, although this is not commonly done nor is it generally necessary (Hunt, 1984).

2.18. THERAPEUTIC MANAGEMENT OF CLINICAL CASES OF CUTANEOUS PAPILLOMATOSIS IN CATTLE

Bovine, equine and canine papillomatosis were successfully treated with formalized homologous or heterologous tissue vaccines injected subcutaneously 3-4 times at 8 (eight) days intervals (Stilinovic, 1955).

The flat warts can be treated with repeated applications of glacial acetic acid or caustic potash-stick and repeated applications of strong solutions of blue stone or even castor or olive oil to multiple small warts on the body of the cattle (Belschner *et al.*, 1972).

A vaccine was prepared from tissue of bovine warts by Diernhfer's method. This was injected subcutaneously in increasing doses, 10, 20, 30 and 40 ml at 7-10 days intervals in 90 affected cattle. Most of the warts dried up and fell off within 2-3 months (Bajric *et al.*, 1974).

Autogenous vaccination can be used as the successful treatment of bovine papillomatosis in Bangladesh than surgical excision and anthiomalin treatment (Ahmed *et al.*, 1978).

The autogenous vaccine used for treatment of generalized cutaneous papillomatosis in cross-bred cattle was found to be effective and useful (Prasad *et al.*, 1980 ; Suveges and Schmidt, 2003). On the contrary, Treatment with autogenous wart vaccine sometimes failed (Smith, 1990). A better response was observed by using autogenous wart vaccine when compared with the response by using anthiomalin, antimosan, Fowler's solution in case of cattle and goats (Rajguru *et al.*, 1988).

Commercial vaccines for cattle rarely seem to effectively promote regression of existing warts or to prevent malignant progression, although they may be capable of preventing the development of new lesions if the same strain is involved (Smith, 1990; Campo, 1991; Scott and Anderson, 1992).

A therapeutic study was conducted on cattle papillomatosis with own blood of the affected cattle as well as with 'Flogocid' ointment but method surgical intervention. It was established that the application of the therapy in 88.8% of the cases resulted in relatively rapid drying of papillae, their regression and curing in relatively short time (Mandic and Perovic, 1986). This referred particularly to heads with papillae located on mammary glands of the skin and udder.

The surgical removal of warts is recommended if the warts are sufficiently objectionable. Surgical intervention in the early growing stage of a wart may lead to recurrence and stimulation of growth. Therefore, it has been suggested that warts should be removed when near the maximum size or when regressing. It was also observed that vaccine of wart tissues containing formalin killed virus have been used for treatment with limited success. Since wart viruses are mostly species specific (Merck, 1986).

Bovine cutaneous papillomatosis or warts occur occasionally in cattle and buffaloes. Variable success has been achieved with allopathic treatment. However, the disease was generally self limiting with spontaneous recovery without treatment (Gupta *et al.*, 1989).

Papilloma of dog on its tongue and mucus membrane of lips was successfully treated with homeopathic drug 'Antimonium crudum' (Dighe, 1992).

Papilloma successfully treated using homeopathic medicine 'Thuja' (Soni *et al.*, 1977).

For the treatment of bovine papillomatosis, autogenous vaccine prepared from warts tissue of affected animals are effective. Cauterization with trichloroacetic acid or 20% tincture of salicylic acid can be used as treatment of warts. The surrounding skin needs to be protected with petroleum jelly. The injection of proprietary preparations containing antimony and bismuth also useful (Radostits *et al.*, 1994).

Both salicylic acid and fig (*Ficus carica*) tree latex were evaluated as having similar therapeutic effects in treating teat papillomatosis in cow (Hemmatzadeh *et al.*, 2003).

Ivermectin, as either single or double dose applications, is effective as a treatment for cutaneous papillomatosis (Atalay *et al.*, 2007).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. EXPERIMENTAL AREA

Bovine cutaneous papillomatosis was studied at Veterinary Teaching Hospital (VTH) of Hajee Mohammad Danesh Science and Technology University (HSTU), surrounding village (Nandoir) of HSTU campus and Birgonj upazila at Dinajpur district of Bangladesh.

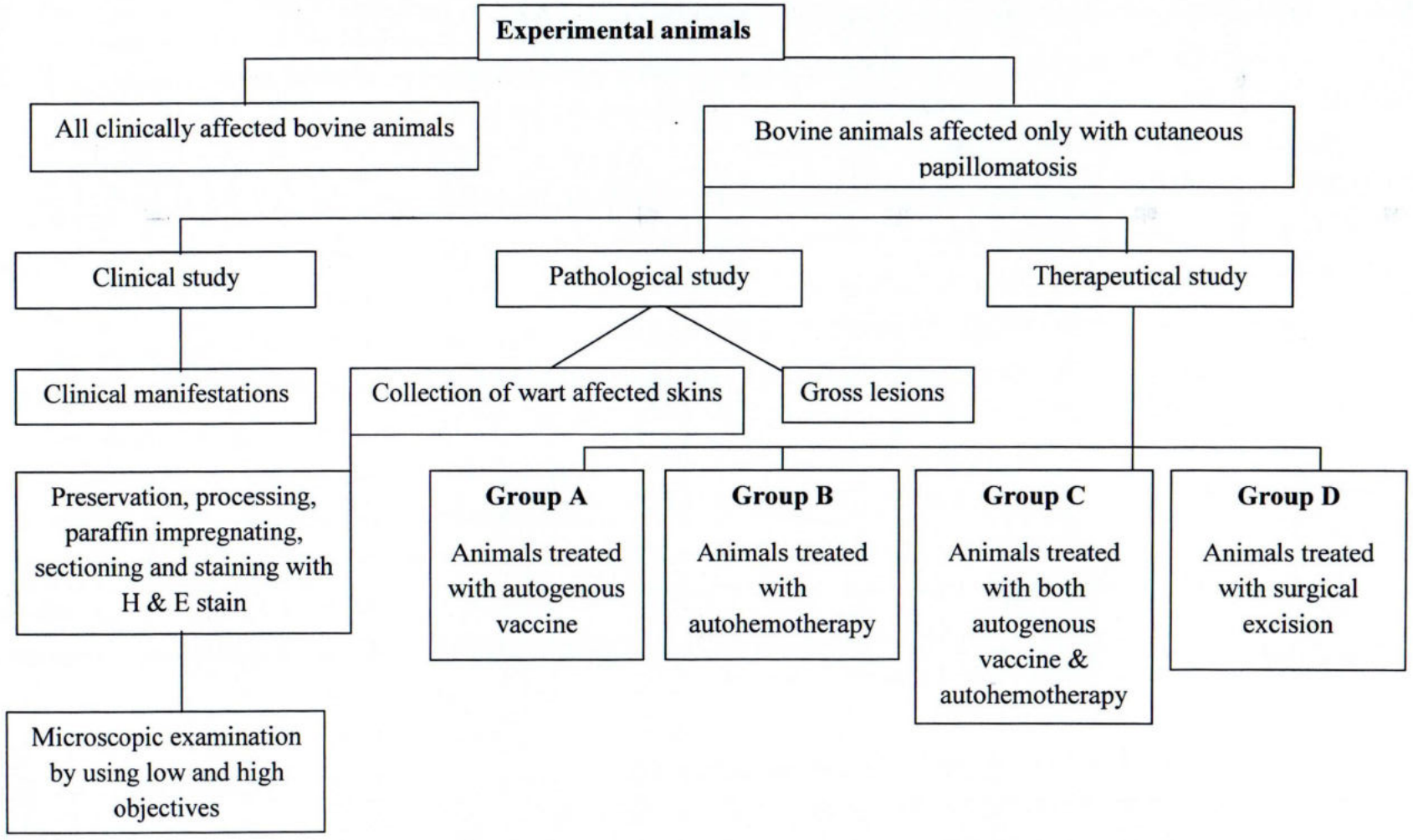
3.2. EXPERIMENTAL ANIMALS

The clinically affected animals visited physically in different locations surrounding the hospital, in Birgonj and at the hospital. The characteristic features and the distribution of all the lesions found in these animals were recorded following the visual and physical examination techniques.

3.3. EXPERIMENTAL DURATION\STUDY PERIOD

The duration of the experiment was one year and conducted from March 2011 to February 2012. The total population of the clinical cases was 886 among which 12 cases of bovine cutaneous papillomatosis were registered in this hospital, surrounding the village and Birgonj during the course of the experimental period.

SCHEMATIC REPRESENTATION OF EXPERIMENTAL DESIGN



3.4. CLINICAL EXAMINATIONS

The presented clinical manifestations of the bovine cutaneous papillomatosis were recorded and the farmer's complaints in relation to the affection were also emphasized. The locations of the lesions, conditions and complications of the cutaneous lesions were recorded.

3.5. PATHOLOGICAL EXAMINATIONS

The gross morbid lesions of the disease were systematically examined, noted and categorized . The suitable sizes of skins with wart lesion of 2 typically cutaneous papillomatosis affected cattle were collected from the live patients subjected for the diagnosis and treatment for further histopathological study.

The representative cutaneous tissues were collected and preserved at 10% formalin solution and subsequently processed, embedded with paraffin, sectioned and stained with haematoxylin and eosin for histopathological examination (Luna, 1968).

3.5.1. Collection of cutaneous papillomatosis affected skin

- Surgical instruments were sterilized by boiling.
- Restraining of animals was performed by casting.
- Local anaesthetic was applied subcutaneously following site selection and waited for few minutes for anaesthetic action.
- Folding of skin was done by artery forceps.
- Excision of excess folded portion of skin and subsequently sutured with nylon threads.
- Locally application of cotton admixing with Tincture of iodine as counter irritant.
- Antibiotic course was maintained and hygienic measures were suggested to avoid secondary complication.
- Suture was removed after 7 days.

3.5.2. Preservation of wart tissues and tissue processing

- Collected wart samples were preserved at 10% formalin solution for at least 3 days.
- Trimming of preserved samples was done at suitable sizes.
- Overnight watering of tissues was done to remove formalin.

- Dehydration was performed in a series of ascending grades of alcohol
  - 50% alcohol: 1hr
  - 70% alcohol: 1hr
  - 80% alcohol: 1hr
  - 95% alcohol: 1hr
  - 100% alcohol: 3 changes and 1 hr for each change
- Chloroform treatment: 2 changes and 1.5 hrs for each change
- Impregnation by paraffinization at melting point (56°C): 2 changes and 1.5 hrs for each change
- The cooked tissue samples were blocked
- Sectioning was done at 5-7µm in thickness, placing on water bath, taking on a glass slide and air dry

3.5.3. Routine haematoxylin and eosin (H & E) staining procedures

Table 2: Preparation of Ehrlich's Haematoxylin solution

Chemicals	Amount
Haematoxylin crystals	4.0 g
Alcohol, 95%	200.0 ml
Potassium or ammonium alum	6.0 g
Distilled water	200.0 ml
Glycerine	200.0 ml
Glacial acetic acid	20.0 ml

Table 3: Preparation of Eosin stock solution

Chemicals	Amount
Eosin Y, water soluble	1.0 g
Distilled water	20.0 ml
Alcohol, 95%	80.0 ml

Table 4: Preparation of Eosin working solution

Chemicals	Amount
Eosin stock solution	1 part
Alcohol, 80%	3 part

0.5 ml glacial acetic acid was added to 100 ml of working eosin solution just before use.

3.5.4. Protocol of haematoxylin and eosin (H & E) staining

- Xylene treatment: 3 changes and 3 minutes for each change
- Rehydration in descending grades of alcohol
  - 100% alcohol: 2 minutes
  - 95% alcohol: 2 minutes
  - 80% alcohol: 2 minutes
  - 70% alcohol: 2 minutes
  - Distilled water: 10 minutes
- Haematoxylin: 10-15 minutes
- Distilled water: 15 minutes
- Bluing in lithium carbonate: Few dips
- Eosin: 30 minutes
- Dehydration in ascending grades of alcohol
  - 80% alcohol: Few dips
  - 95% alcohol: Few dips
  - 100% alcohol: Few dips
- Xylene treatment: 3 changes and 3 minutes for each changes
- Mounting with Canada Balsam
- Examined under microscope using both low and high power objectives

3.6. THERAPEUTICAL FINDINGS

The therapeutic strategies were categorized into 4 groups among which Group A received autogenous vaccine; Group B received autohaemotherapy; Group C received both autogenous vaccine and autohaemotherapy and in Group D, surgical excision was given.

A total of 12 (twelve) cattle affected severely with typical lesions of cutaneous papillomatosis were selected for 4 (four) different therapeutic trials.

Group - A

Consisted of four (4) clinical cases having mostly cauliflowers and pedunculated types of lesions on different regions of the body surface. They were subjected to therapeutic trials with autogenous vaccines. About 10 ml of the vaccines were injected subcutaneously at the neck region to each animal and it was repeated for five occasions at an interval of seven days. Disposable syringe and needles were used for this purpose. Before each repeatation of injection, the lesions were examined for observing regressing signs if any. The autogenous vaccines were prepared following the technique of Ahmed *et al.* (1978) which has been described at the end of this chapter.

Group - B

Consisted of 3 (three) clinical cases. These animals were treated with their own blood (auto-haemotherapy) as described by Mandic and Peruvic (1986) and Rahman (1996). About 15 ml of blood was collected aseptically from the jugular vein of each animal and injected immediately into the same animal intramuscularly at the thigh muscles. The same dose was repeated for four occasions at an interval of 5 days depending on the degree of responses to the treatment.

Group - C

Consisted of 3 (three) clinical cases characterized by severe multiple pea sized, cauliflowers and pedunculated type lesions on the face, abdominal and fore limb regions. They were given treatment with both of the autogenous vaccine and auto-haemotherapy at 7 days interval. The dose was repeated for four occasions.

Group – D

This group consisted of two cattle affected with both cauliflower and pedunculated types of lesions at neck region. Surgical excision was given to this group. First, restraining of animals was performed by casting. Then local anaesthetic was applied subcutaneously and waited for few minutes for anaesthetic action. Base of wart tissue grabbed by artery forceps and excision was done and subsequently sutured with nylon threads. Then locally application of cotton admixing with Tincture of iodine as counter irritant was used. Antibiotic course was maintained and hygienic measures were suggested to avoid secondary complication. Suture was removed after 7 days.

3.7. PREPARATION OF AUTOGENOUS VACCINE

About 2 gm of wart tissue was excised aseptically from some old lesions. This was triturated in a morter with pestle adding about 10ml of normal saline. It was then first filtered through 2-3 layers of gauge cloths to remove the course particles. After this the filtrate was again filtered through filter paper. To each 10ml filtrate were added penicillin 10,0000 unit, streptomycin 250 mg and formalin 1 drop. The material was left at room temperature for about 40 minutes after which 5-10 ml of materials was injected subcutaneously (Ahmed *et al.*, 1978).

3.8. PHOTOGRAPHY

The histopathological slides of normal and wart affected cutaneous tissues were placed in microscope (Leica, Germany) and the respective microphotographs were taken directly by a digital camera (SONY DSC-W520, 14.1 MEGA PIXEL, China) using both low and high power objectives (X4, X10 and X40). The photographs were then placed in computer, image selection and magnification were further modified and placed in this thesis for better illustration of the results.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1. CLINICAL MANIFESTATIONS

The patients affected with cutaneous papillomatosis could be ranged from moderate to severe types. The demarcation between moderate and severe stage of the disease could be made based on the degree of severity, level of dissemination of lesions, complication or concurrent infections. Characteristic clinical features were pedunculated cauliflower like, sessile and pea shaped lesions throughout the skin. These lesions of the disease were diagnosed entirely on the basis of clinical grounds mainly by visual examination and palpation.

Table 5: Prevalence of bovine cutaneous papillomatosis

Places	No. of animals examined	No. of animals affected with wart	Percentage (%) of wart affected animal
Veterinary Teaching Hospital (HSTU)	456	4	0.88
Nandoir	190	3	1.58
Birgonj	240	5	2.08
Total	886	12	1.35

4.2. GROSS LESIONS

In the present study, there were three main types of lesions observed in clinical cases under field condition. Characteristic lesion of cutaneous papillomatosis in cattle has been shown in Table-6. These were pedunculated cauliflower like, sessile and pea shaped lesions. This study include 12 affected animals. Pedunculated lesions was recorded highest (50%), followed by pea/bean shape (25%) and sessile type lesions (8.33%). Warts are solid outgrowth of epidermis and some of the lesions were pedunculated. The sessile and pea shaped lesions were in different sizes from 1-2 cm upwards and found in the form of dry horny appearance under magnifying glass.

The distribution of lesions and number of lesions at different sites of affected animals have been shown in Table-7. The more usual sites were the neck (25%), shoulder (25%), face (16.67%), around the eyes (16.67%), fore legs (16.67%), hind legs (8.33%), abdominal wall (8.33%), costal area (8.33%), around the udder (8.33%) and around the genitalia (8.33%). The average number of lesions was highest in neck and shoulder area.

Table 6: Characteristic gross lesion of cutaneous papillomatosis in cattle.

Sl. No.	Lesions	Animals affected	
		No.	%
1	Pedunculated cauliflower like	6	50%
2	Pea / bean shape	3	25%
3	Sessile	1	8.33%
4	Others	2	16.67%
Total		12	100

Table 7: Distribution of cutaneous papillomatous lesions in different body regions in cattle.

Sl. No.	Affected body region	Animals affected (Total =12)	
		No.	%
1	Neck	3	25
2	Shoulder	3	25
3	Face	2	16.67
4	Around the eye	2	16.67
5	Fore legs	2	16.67
6	Hind legs	1	8.33
7	Abdominal wall	1	8.33
8	Costal area	1	8.33
9	Around the udder	1	8.33
10	Around genitalia	1	8.33

4.3. HISTOLOGICAL FEATURES

The wart tissue of the cutaneous papillomatosis affected patient submitted for diagnosis and treatment were collected, preserved, processed and stained for histopathological observation. The characteristic histopathological lesions of the bovine cutaneous papillomatosis were thickening in the layer of stratum corneum of epidermis (hyperkeratosis), thickening of stratum granulosum, thickening of stratum spinosum (acanthosis) and downgrowth rete ridges which extend between dermal papillae. The continuity of superficial layer of epidermis was lost. Fibrous connective tissue proliferation and islands of neoplastic cells were also found. Histopathological features of the spinous cells of the squamous epithelium present with hyperchromatic nuclei surrounded by a perinuclear clear zone (koilocytosis).

4.4. THERAPEUTICAL FINDINGS

The results of the different therapeutic trials conducted against bovine cutaneous papillomatosis has been shown in Table-8.

Group A : In this group one animal out of four cattle treated with autogenous vaccines showed gradual regression of the wart lesions after the third injection and recovered almost completely (25%) after the fifth injection within a total period of about 2 months. One animal (25%) of this group recovered partially to the treatment. Two animals (50%) did not respond to the treatment.

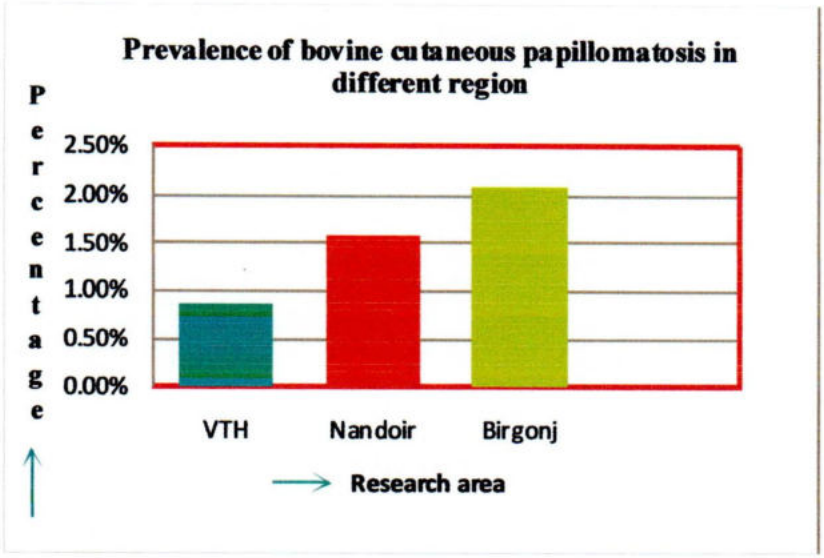
Group B : A total of three animals were treated with their own blood out of which one animal (33.33%) did not respond completely but showed some regression of the lesion after the fourth dose. Two animals (66.67%) did not respond to the treatment.

Group C : A total of three animals received both autogenous vaccine and autohemotherapy. Two animals cured partially (66.67%) after fourth injection and one animal (33.33%) did not cure.

Group D : In this group surgical excision was given to the two animals. All of them were completely (100%) cured after 20 days.

Table 8. Comparative responses to the different therapeutic trials against cutaneous papillomatosis in cattle.

Groups	Types of therapy	No. of animals treated	Therapeutic responses		
			Completely cured	Partially cured	Treatment failure
Group A	Autogenous vaccine	4	1 (25%)	1 (25%)	2 (50%)
Group B	Autohemotherapy	3	0	1 (33.33%)	2 (66.67%)
Group C	Combination of Autogenous vaccine & Autohemotherapy	3	0	2 (66.67%)	1 (33.33%)
Group D	Surgical excision	2	2 (100%)	-	-
Total		12	3 (25%)	4 (33.33%)	5 (41.67%)



*VTH = Veterinary Teaching Hospital

Figure 1: Graphical representation of prevalence of bovine cutaneous papillomatosis.

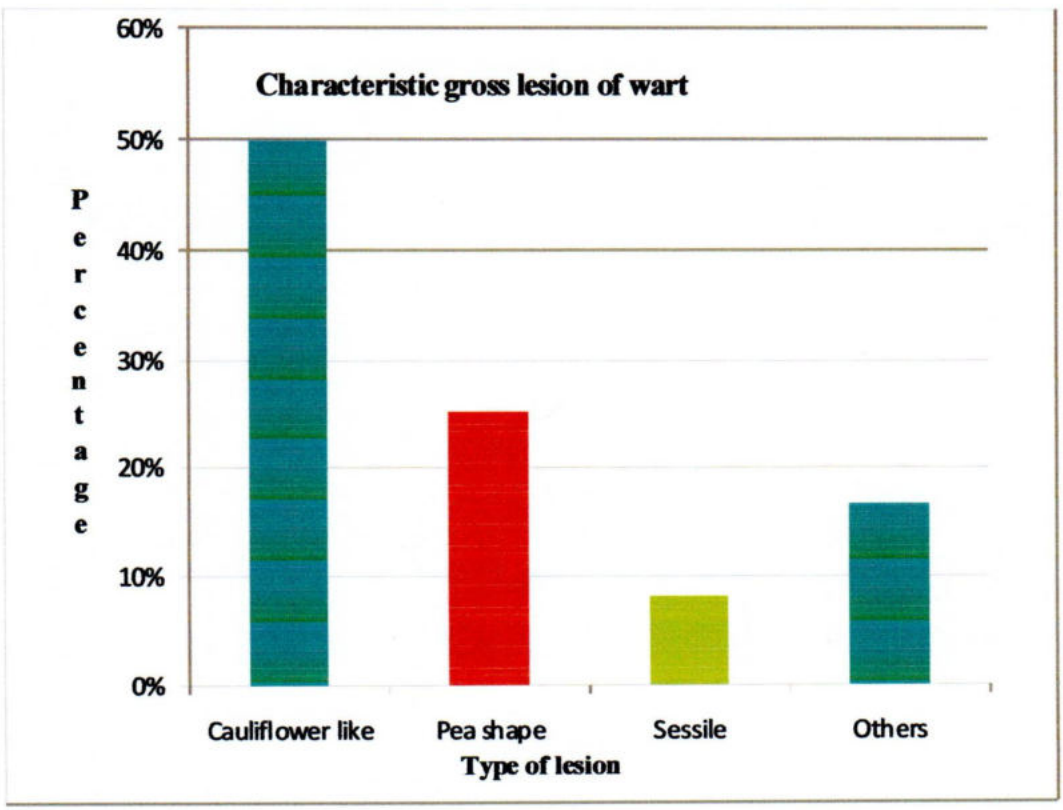


Figure 2: Graphical representation of various types of wart lesion found in cattle.

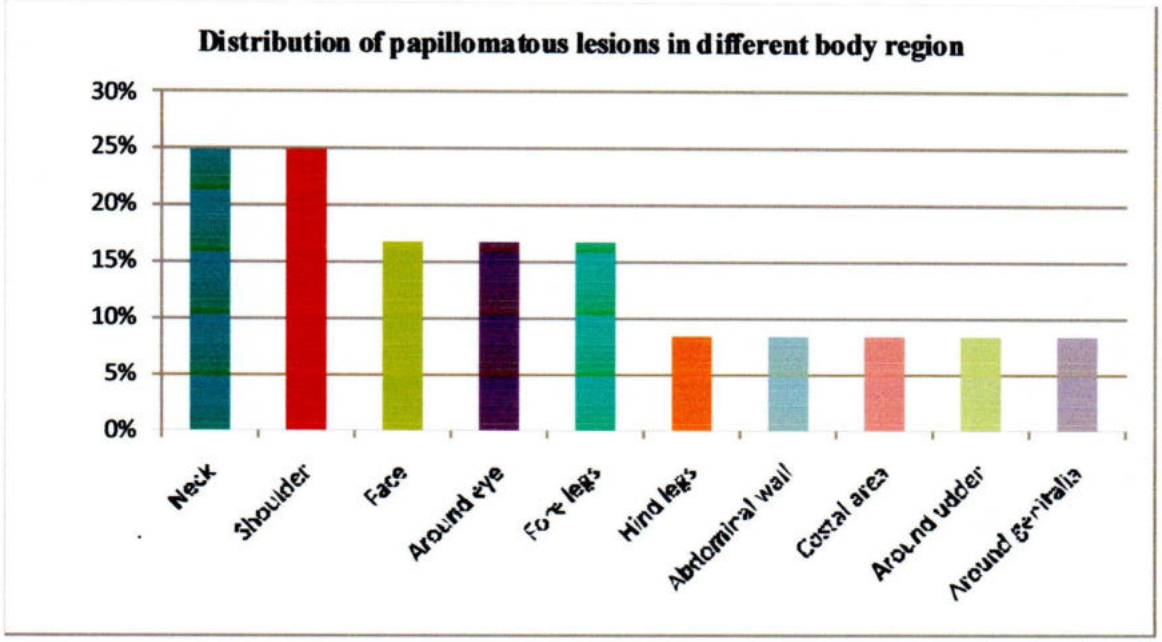


Figure 3: Graphical representation of distribution of lesion in different body parts of cattle.

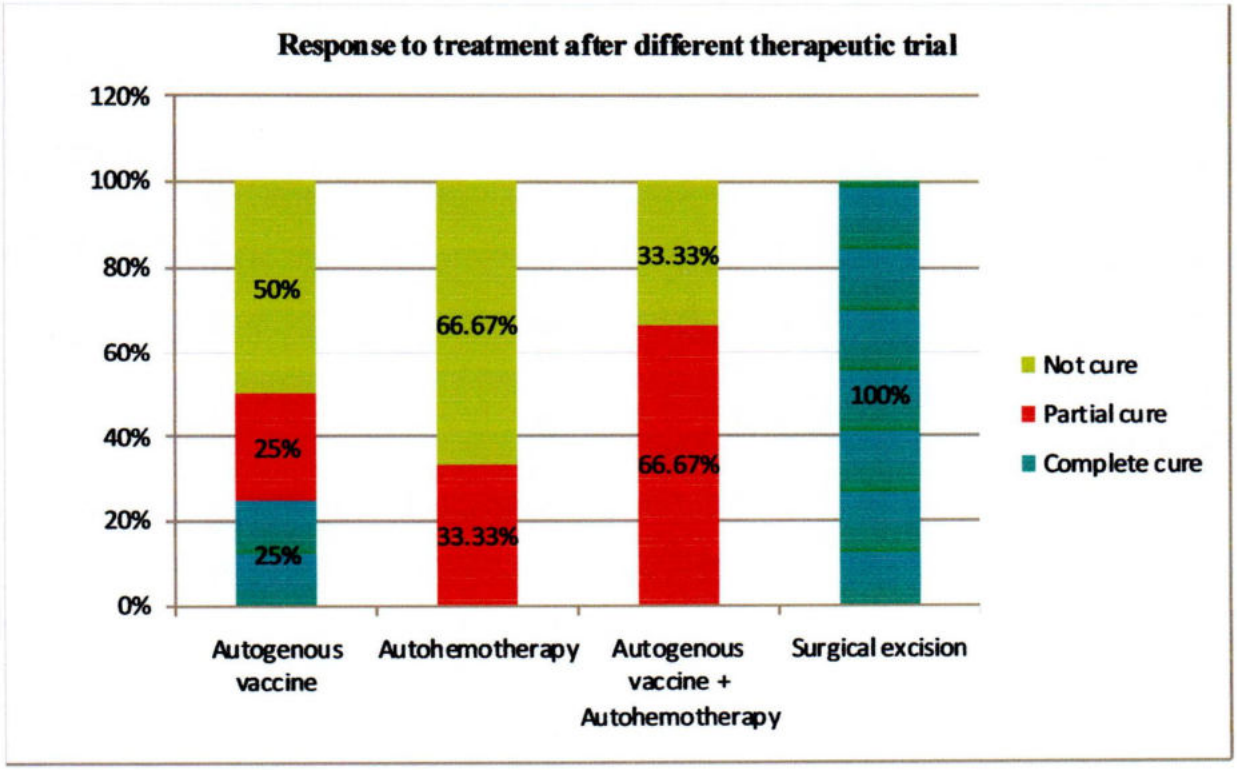


Figure 4: Graphical representation of response to treatment after different therapeutic trial of wart affected animals.

Topographical distribution of lesions at different body region

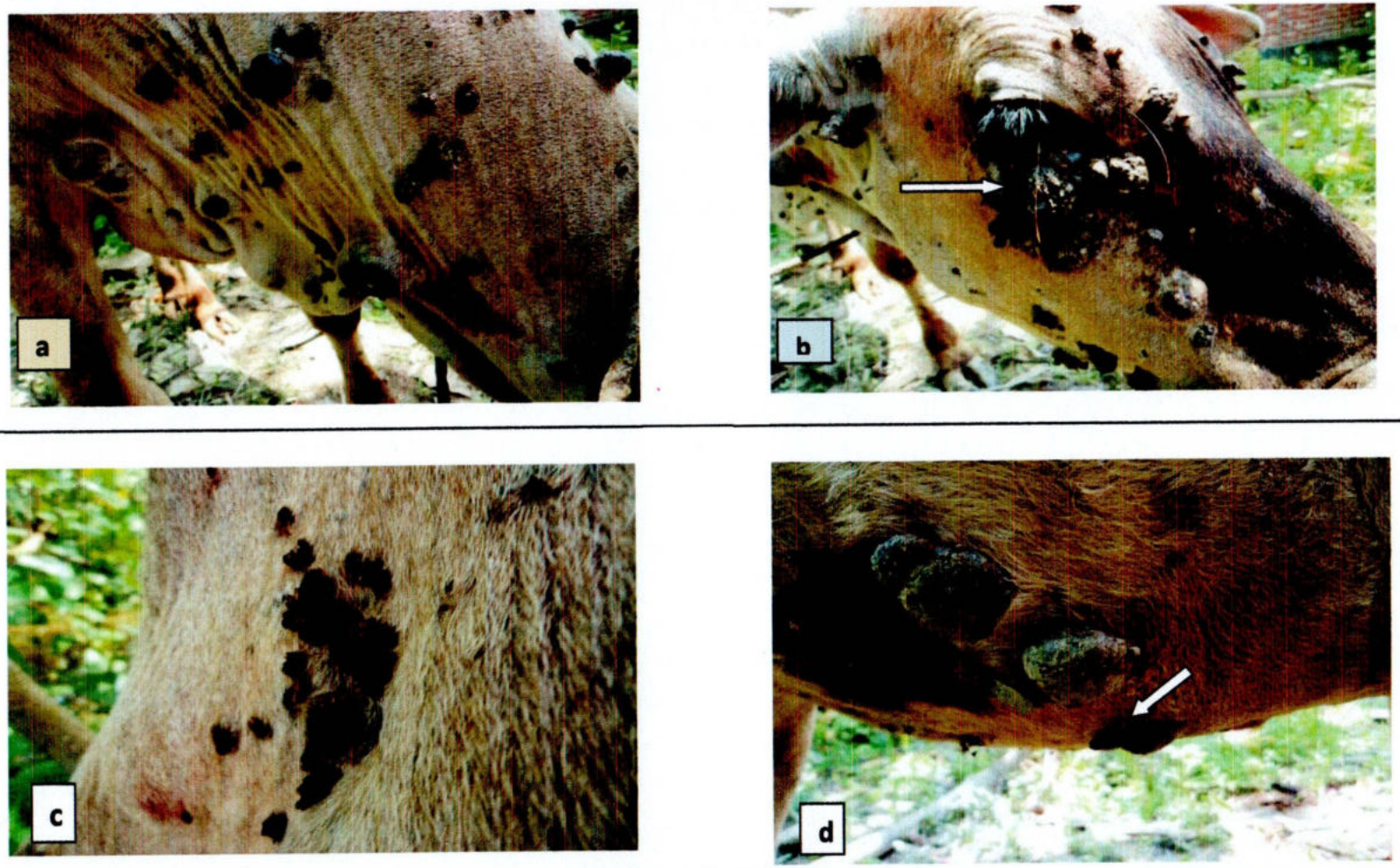


Figure 5: Characteristic gross lesions at different body parts: (a) Shows wart lesion at neck region, (b) Cauliflower like lesion at lower eyelid area (in arrow), (c) Wart lesion at shoulder area and (d) arrow shows pea shaped lesion at abdominal region.

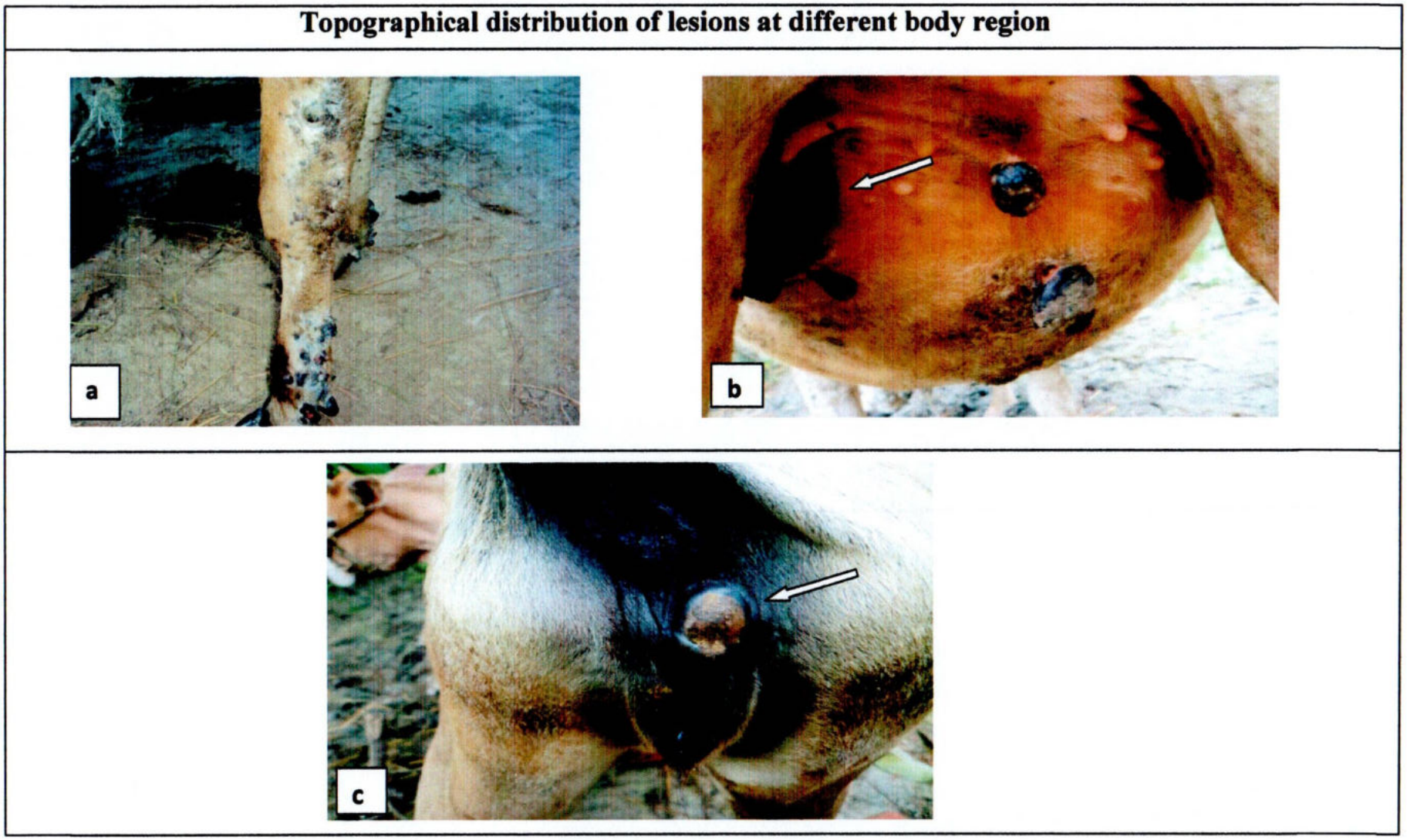


Figure 6: Cutaneous papillomatous lesions : (a) fore limb region, (b) Udder area (arrow shows pedunculated lesion), (c) Genital area (in arrow).

Therapeutic responses after different therapeutic trial

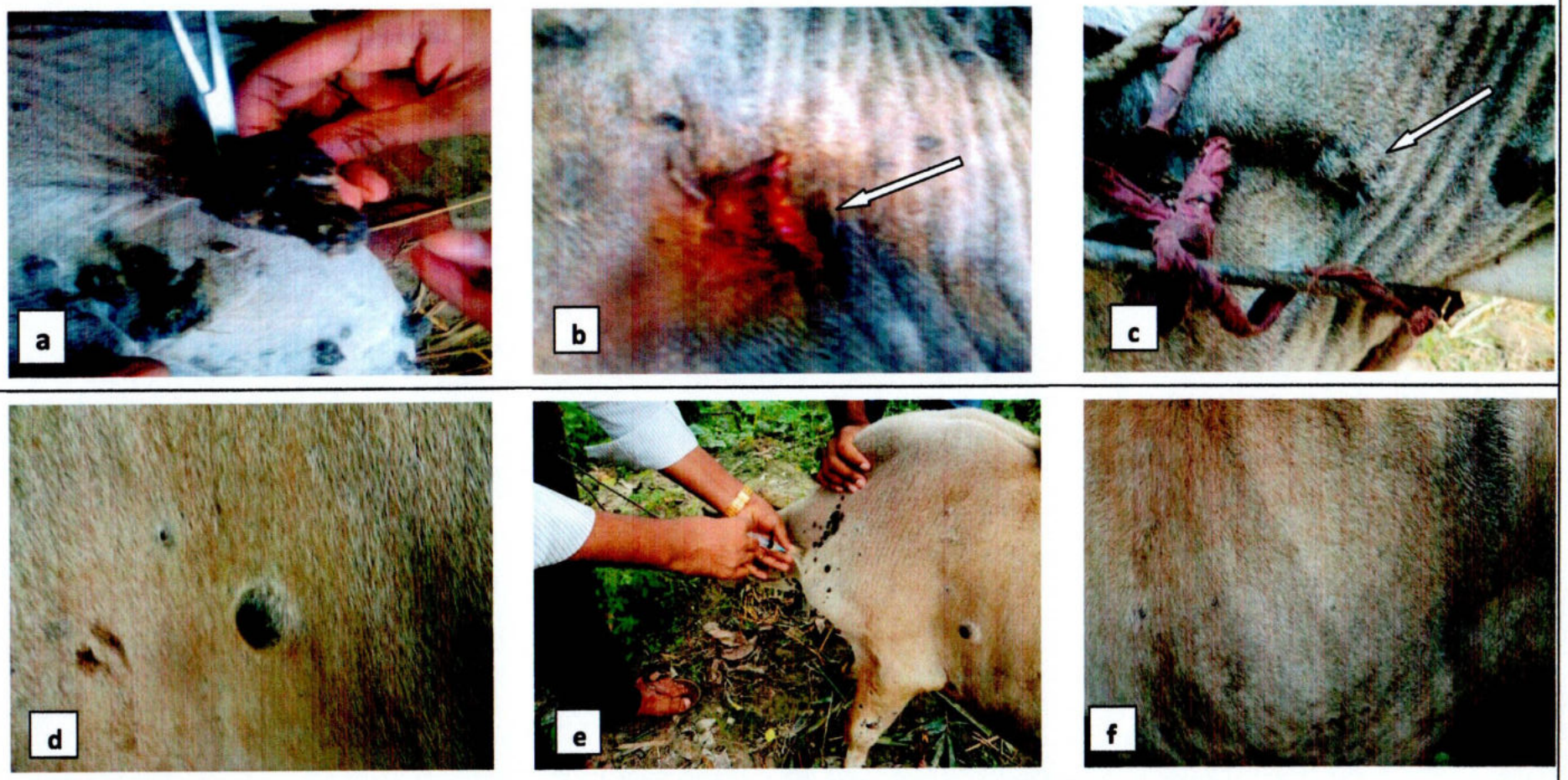


Figure 7: Therapeutic responses: (a) During surgical excision of wart lesion, (b) after surgical excision, (c) 2 weeks after surgical excision (in arrow), (d) shows wart lesion before autogenous vaccination (e) autogenous vaccination at subcutaneous area, (f) after autogenous vaccination.

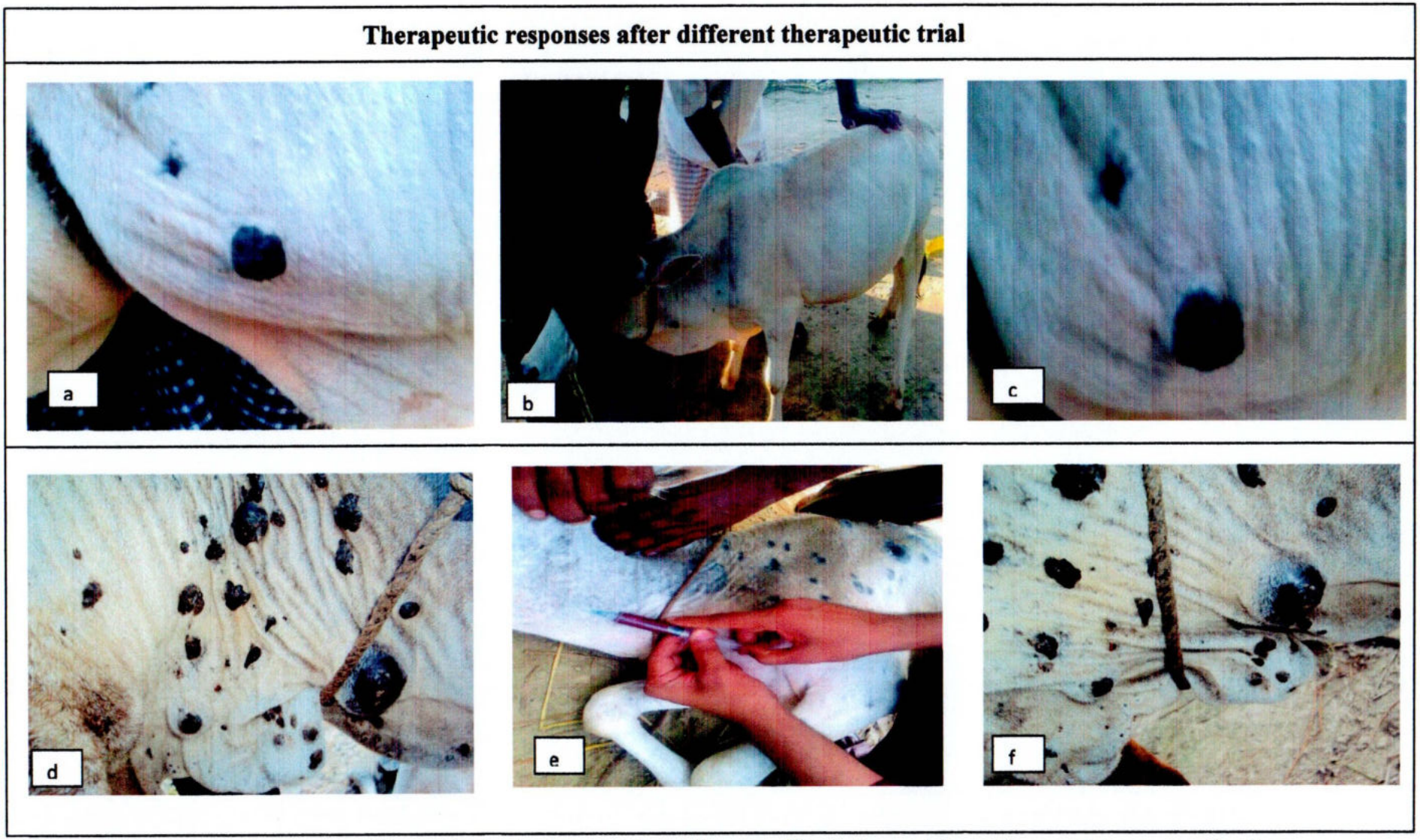


Figure 8: Therapeutic responses: (a) wart lesion before autohemotherapy, (b) during autohemoterapy, (c) one month after autohemotherapy, (d) wart lesion before combined therapy of autogenous vaccination and autohemotherapy, (e) during therapy (f) after 4th doses of therapy.

Histopathology of bovine cutaneous papillomatosis

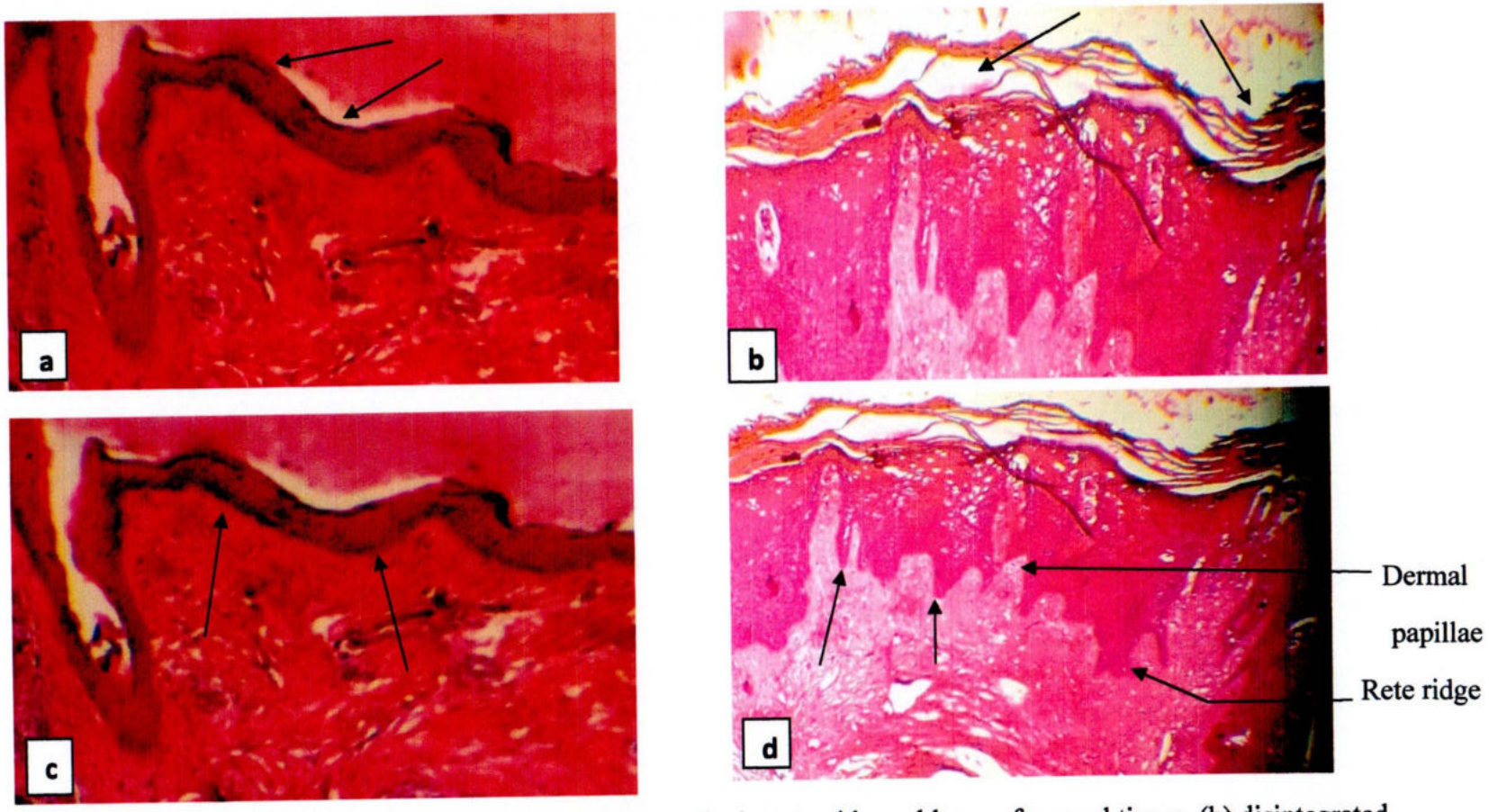


Figure 9: Histopathological features of wart: (a) arrows indicating the intact epidermal layer of normal tissue, (b) disintegrated superficial layer of epidermis of a wart tissue (in arrows), (c) in arrows, shows no epidermal layer thickening (normal tissue), (d) epidermal thickening that extend downward between dermal papillae which called 'rete ridges' found in a wart tissue.

Histopathology of bovine cutaneous papillomatosis

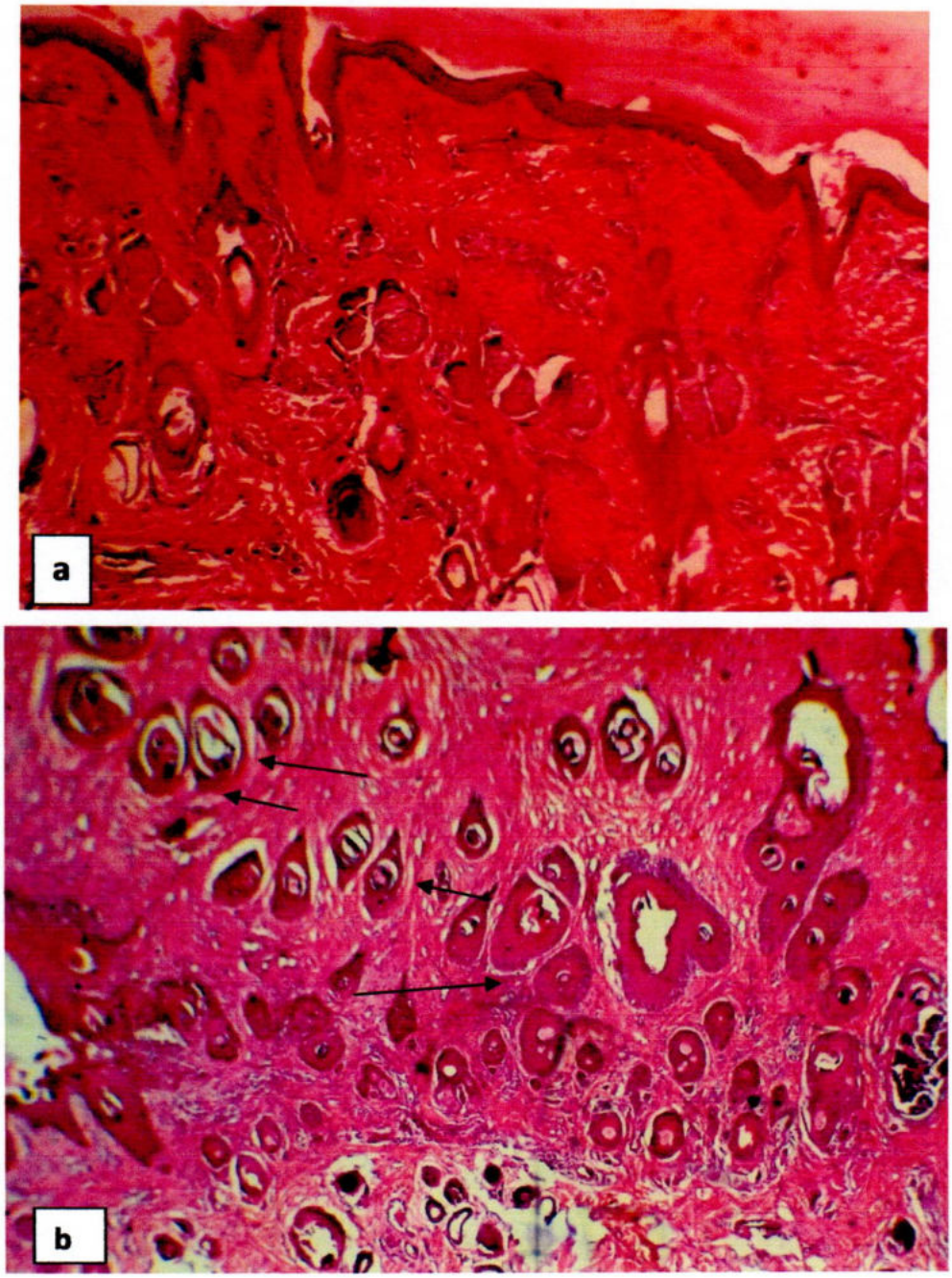


Figure 10: Histopathological features: (a) normal tissue shows no neoplastic cell proliferation, (b) arrows show neoplastic cell islands of a wart tissue.

Histopathology of bovine cutaneous papillomatosis

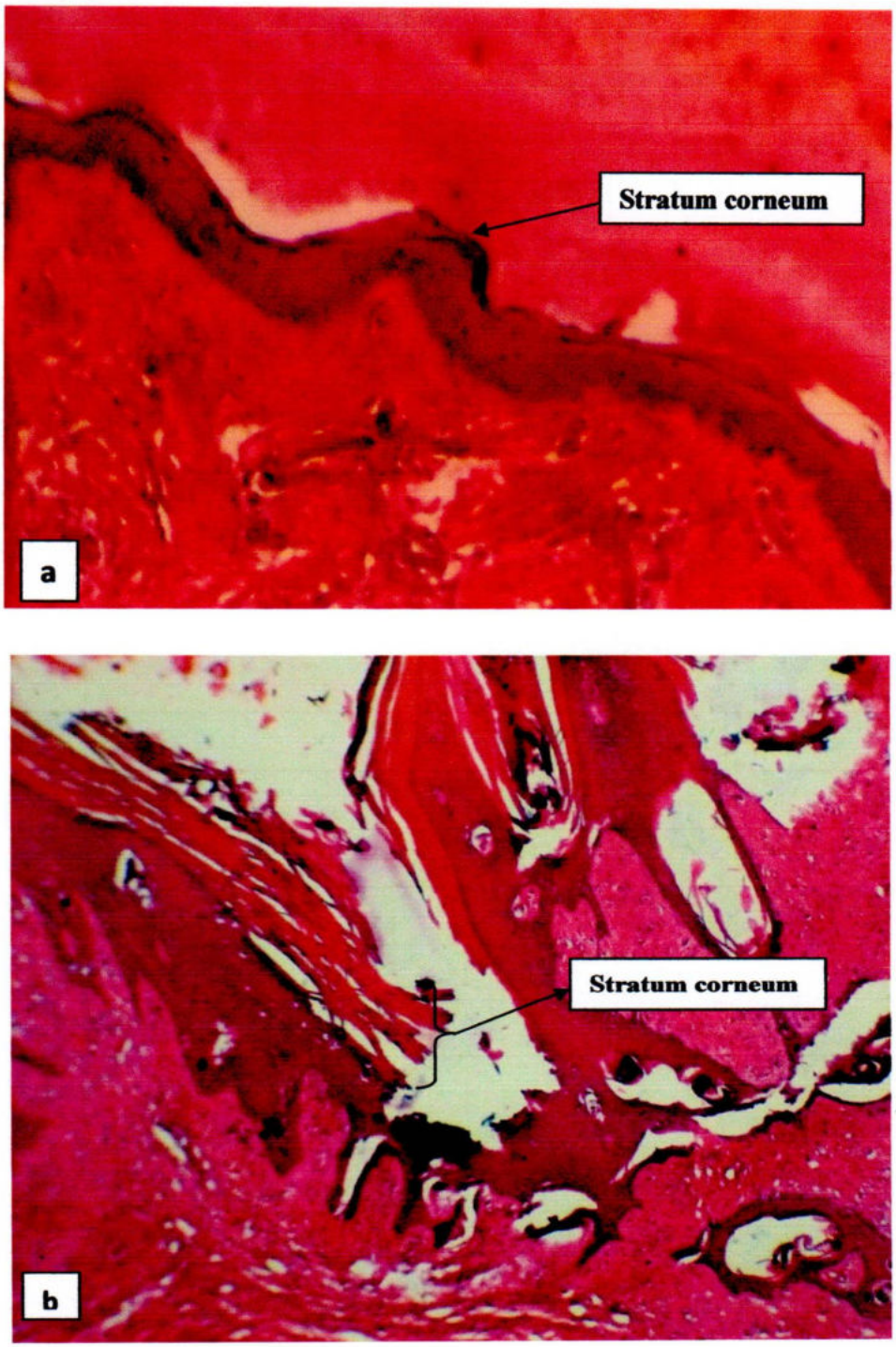


Figure 11: Histopathological features: (a) there is no thickening of stratum corneum of epidermis (arrow) of a normal tissue, (b) shows marked thickening of stratum corneum (Hyperkeratosis) of a wart tissue.

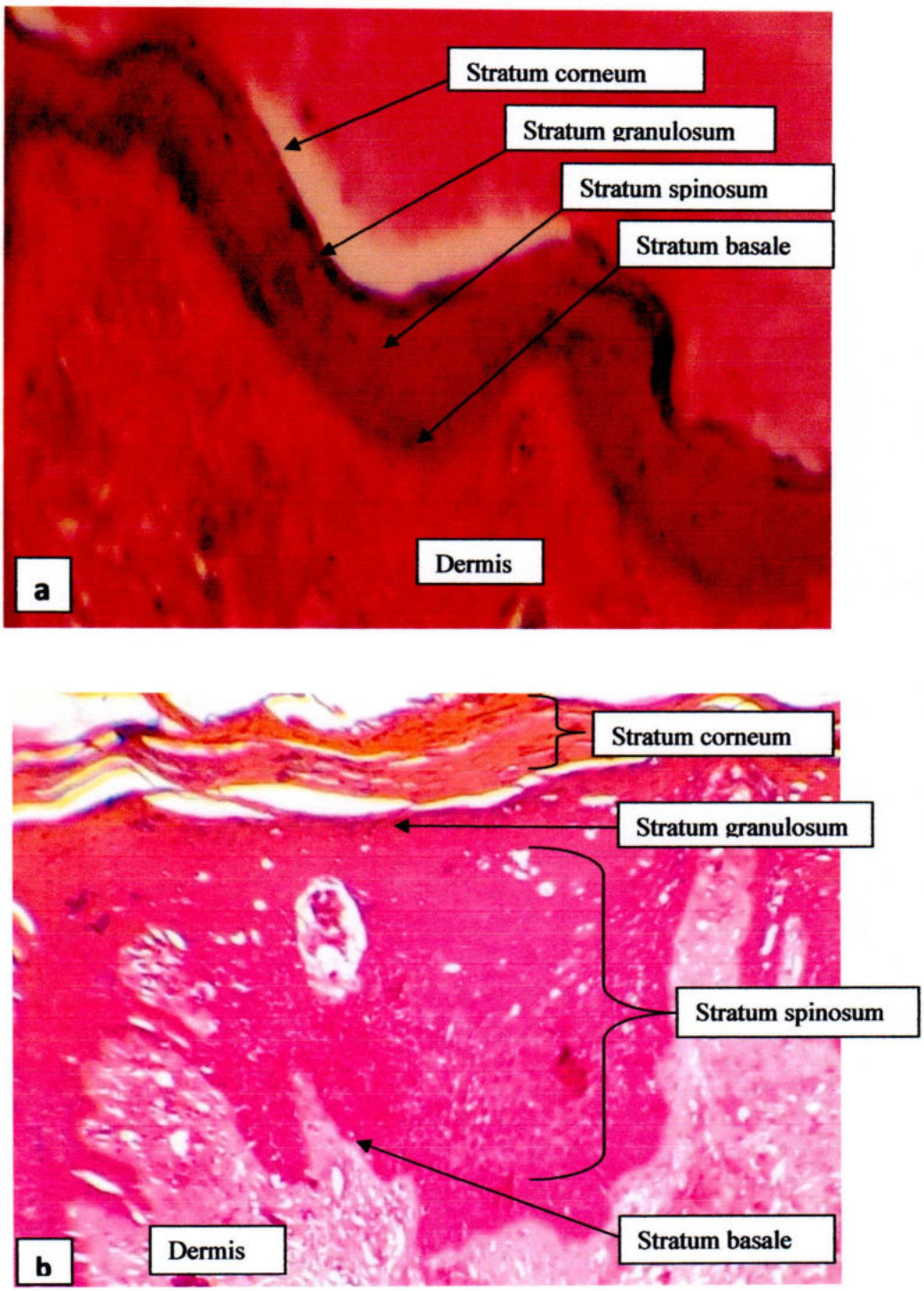


Figure 12: Histopathological features: (a) shows different layer of epithelium of a normal skin of cattle, (b) shows thickening of stratum corneum (hyperkeratosis), thickening of stratum granulosum, thickening of stratum spinosum (acanthosis) of a wart tissue.

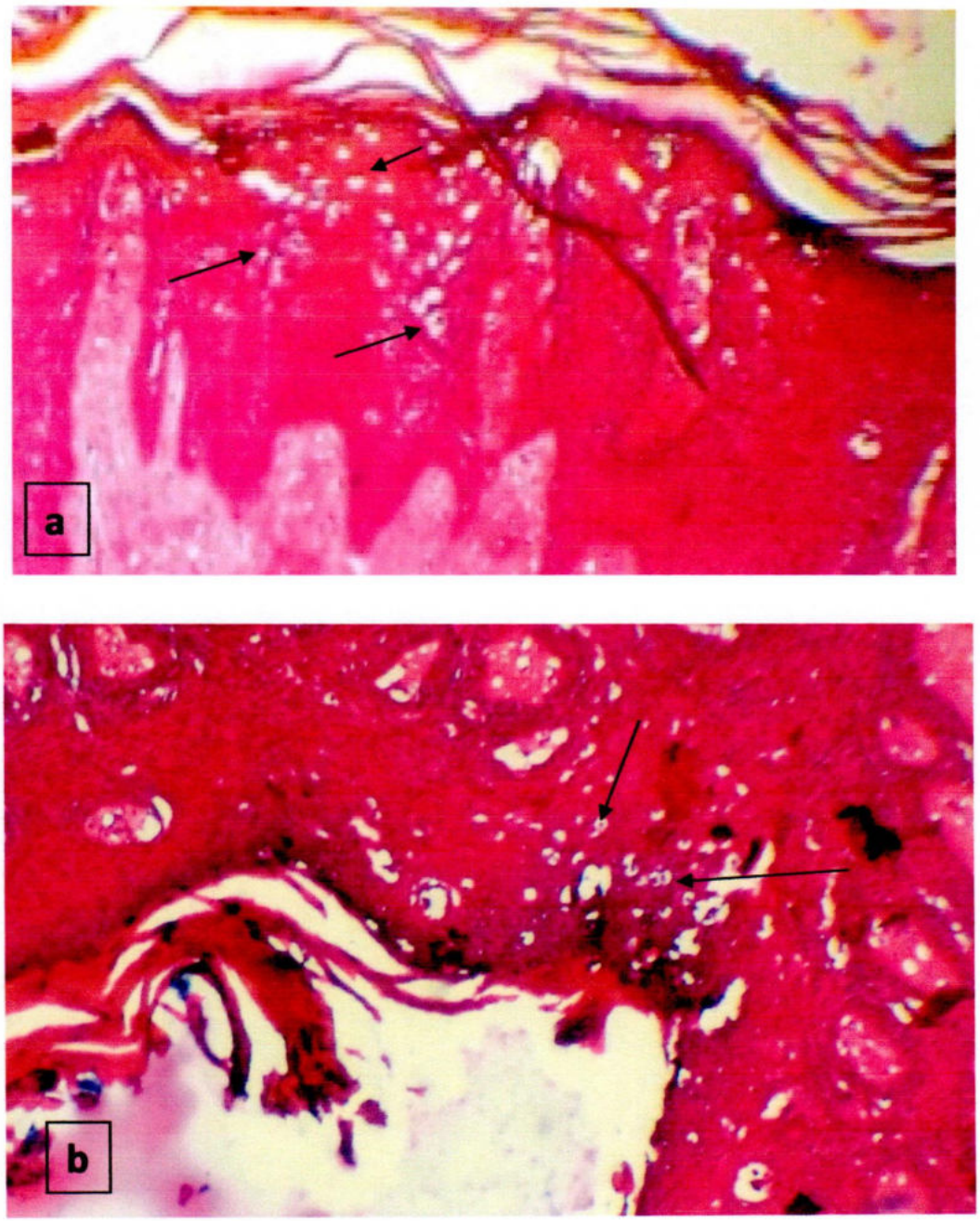


Figure 13: Histopathological features of spinous cells of epithelium: (a) & (b) shows enlarged (2 to 3 times more than normal) and hyperchromatic nuclei. A clear area surrounding the nucleus, known as perinuclear halos (in arrows). Total term is known as koilocytosis.

Histopathology of bovine cutaneous papillomatosis

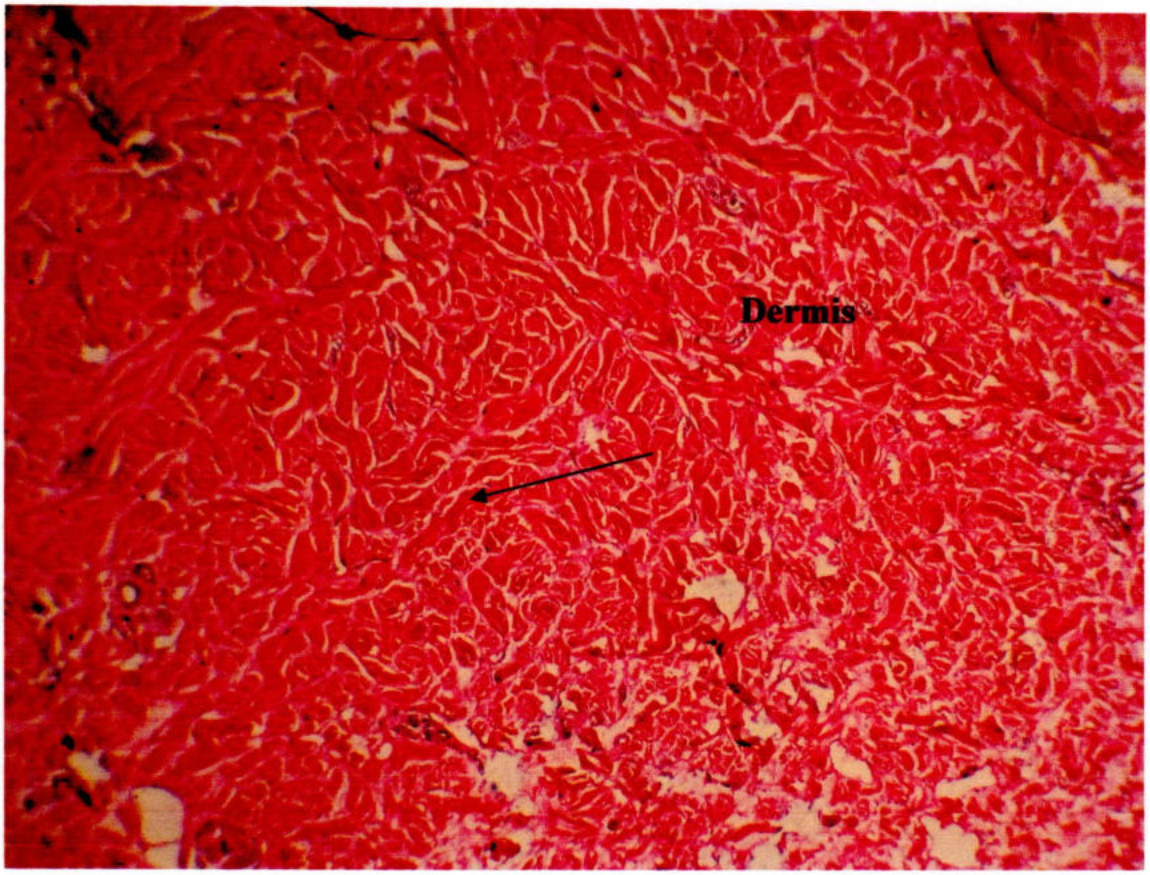


Figure 14: Histopathological features: arrow shows fibrous connective tissue proliferation especially collagen fibers at dermal region of papillomatosis affected skin.

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

5.1. CLINICAL INCIDENCE

During the period of February, 2011 to March 2012, a total of 886 cattle were examined and out of them 12 animals had typical lesions indicating 1.35% general incidence of the disease. This result does not simulate with the earlier reports of Mia and Haque (1967) who reported only 0.29% incidence of the disease out of 6929 cattle in Mymensingh district. The prevalence rate of the disease in Birgonj were higher than that of other places under this experiment. Similar research works were carried out by many workers in other countries with variable results. Radostits *et al.* (1994) reported 25% incidence in pure-bred animals, Rosenberger (1941) reported 2.51% of incidence of the disease in cattle from Germany and Bagdonas *et al.* (1953) 74.5% from USA. This variation might be due to difference of geographical location, management and error in the method of examination.

5.2. GROSS LESION

In this study the characteristic gross lesions were pedunculated, cauliflower like (6), pea or bean size (3) and sessile (1). But different atypical lesions were also found in 2 animals out of 12 clinical cases. Some of these were flat, finger like projection and rice grain sized. In some cases particularly the pedunculated lesions were found lacerated, bleeded and soiled with dung. This findings correlates with that of Huck (1965). The Characteristic lesions of cutaneous papillomatosis of cattle presented in this study similar to those described by Steele-Bodger and Wright (1959), Huck (1965), Rahman *et al.* (1969), Garita (1979) and Barnes (2009).

5.3. DISTRIBUTION OF LESION

Tumor formations were multiple, they can be generalized in almost all body areas, having a typical papilloma appearance, of variable sizes, from 1–2 cm to large structures. In this study the predilection sites of wart lesions were found on the neck, followed by shoulder, face, around the eye, fore legs, costal region, udder and around genitalia which is agreement with the reports of Wirsching (1913), Liess (1934), Cook and Olson (1951), Rahman *et al.*(1969), Das (1982), El-Mahdi *et al.* (1990) and Turk *et al.* (2005).

5.4. HISTOPATHOLOGICAL EXAMINATION

Samples of wart tissue were collected, preserved, processed and stained with hematoxyline and eosin stain for examination under light microscope. There was marked hyperkeratosis of stratum corneum of epidermis (fig-11.b) and discontinuity or loss of integrity of superficial layer of epidermis (fig-9.b). There was marked hyperplasia in the cells of stratum spinosum (acanthosis). These findings of this study correlate with the findings of Hamada *et al.* (1990), Bloch *et al.* (1994) and Eisa *et al.* (2000).

Thickened epidermal layer goes downward in between the dermal papillae which is known as rete ridges (fig-9.d) also found in this study which revealed with the study of Turk *et al.* (2005). The koilocytosis consists of opaque nucleus, hyperchromatic and irregular surrounded by perinuclear halos (Fig-13.a & b) which simulate with the study of Silveria *et al.* (2005). Other author affirms that the koilocytosis constitutes a sign of pathognomonic infection by papillomavirus (Xavier *et al.*, 2005).

There was an extensive proliferation of fibrous connective tissue (non neoplastic) at the reticular area of dermis (Fig-14). Some slides showed characteristic neoplastic cell islands (Fig-10.b). These statement correlate with the findings of Goldschimidt *et al.* (1988). Other important features were not possible to study due to lack of electron microscope and laboratory facilities.

5.5. THERAPEUTIC RESPONSES

Spontaneous remission of papillomas is reportedly uncommon, and regression of papillomas may take almost a year (Olson and others 1992, Campo and others 1994, Smith 1996). Several methods for the treatment of the disease have been recommended; however, there has been no agreement on the best method (Or and Bakırel 2002, Çimtay and others 2003, Hemmatzadeh and others 2003). The treatments of papillomavirus infections are harder and induce to economic loss with excessive spending on drugs without therapeutic success. The affected individuals were categorized into four major groups (Group A, B, C and D). The therapeutic efficiencies have been confirmed either by the physical visits and telephone message.

The therapeutic management of clinical cases of cutaneous papillomatosis with autogenous vaccine was done at the dose rate of 10 ml subcutaneously to the Group-A. In this study, out of 4 animals only 1 animal (25%) completely cured, 1 animal partially cured whereas 2 animals did not response after 5th dose of treatment. Cases of nonregressing canine papillomatosis have been reported after over 10 attempts included with autogenous vaccine (Nicholls *et al.* 1999). Some authors found excellent result on using autogenous vaccination against bovine cutaneous papillomatosis (Stilinovic, 1955; Bajric *et al.*,1974; Ahmed *et al.*,1978; Prasad *et al.*, 1980; Merck, 1986; Rajguru *et al.*, 1988; Radostits *et al.*, 1994; Lesnik *et al.*,1999; Suveges and Schmidt, 2003 and Turk *et al.*, 2005). On the contrary, treatment with autogenous wart vaccine sometimes failed (Smith, 1990).

In this study 33.33% animal showed partial regression of wart lesion clinically after 4th dose of autohemotherapy. Complete regression did not occur may be due to immune system did not work properly and lack of continuation of the doses of autohemotherapy furthermore. However, Mandic and Perovic (1986) reported that 88.8% of the cases resulted in relatively rapid drying of papillae, their regression and curing in relatively short time after autohemotherapy. This referred particularly to heads with papillae located on mammary glands of the skin and udder.

In 3rd group combined use of both autogenous and autohemotherapy was done to 3 animals. Out of these 2 animals (66.67%) showed partial regression of wart. All of animals in this group were

severely affected with papillomatous lesion. Though this findings may bring satisfactory result if the therapeutic doses continued onwards.

Treatment with surgical excision has been recorded as an excellent achievement in Group-D. In this study, the percentage of recovery has been found very high (100%). Healing is rapid and the animals should be show-eligible in a few days (Morter and Horstman, 2007). Surgical excision of warts is a better treatment option for bovine papillomatosis than curetting, autogenous vaccine and administration of levamisole (Fayez and Haithm, 2011). Surgical excision of wart lesion also recommended by Merck (1986).

CHAPTER VI

CONCLUSIONS

CHAPTER VI

SUMMARY AND CONCLUSION

Dermatopathology in respect to bovine cutaneous papillomatosis with clinical observation was studied clinically, pathologically and therapeutically. Clinically the types of lesions observed and recorded as pedunculated cauliflower, sessile, pea / bean shaped and others. The distribution and the predilaction sites of lesions were investigated in the severely affected animals. The predilection sites were neck, shoulder, face, fore legs, around the eyes, abdominal wall, dewlap, around the udder and around genitalia. Histopathologically, the disease was characterised as hyperkeratosis, acanthosis, hypergranulosis, downgrowth of rete ridges and formation of neoplastic cells islands. A total of 12 affected cattle with typical lesions were included for therapeutic trials in separate groups with different formulations and strategy among which surgical excision produce an excellent results (100% recovery), 25% recovery rate after autogenous vaccination and animals recover partially after the treatment with autohemotherapy and combined use of autogenous vaccine and autohemotherapy. Though the disease under the study did not cause loss for death but it causes economic loss from reducing market value of affected animals and their hides and skin. Field veterinarians face this disease problem in frequently. The results of different therapeutic trials conducted under this study will be helpful for the veterinary clinicians in selecting the line of treatment of cutaneous papillomatosis in cattle. However, further studies are needed to be explored to determine cellular involvement for the initiation of papillomatous growth and standard therapeutic protocols.

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